

## ***InsituPro VS***

### **Manual**





## INTAVIS Bioanalytical Instruments AG

develop and provide advanced automated systems for molecular biology and biochemistry. We concentrate on customer-oriented automation of complex protocols in **peptide synthesis, proteomics and functional genomics**.

Our products already support scientists around the world to cope with:

◆ **automated peptide synthesis**

No matter if used as substrates in enzyme assays or for production and purification of antibodies – peptides always play an important role in research and diagnostics. For efficient solid phase synthesis of peptides we offer the instruments **ResPep XL** and **MultiPep**. Miniaturized methods in biochemistry and immunology increase the demand for peptide arrays. The **AutoSpot** is a unique instrument for synthesis of peptide arrays on membranes. Full automation of this process is possible with our **MultiPep SPOT** kit.

◆ **automated protein digestion**

A major step in protein analysis is the chemical or enzymatic fragmentation of proteins separated by gel electrophoresis. The **DigestPro MS** fully automates all steps of this tedious and complex method. You need further automation? Besides offering automated digestion the **DigestPro MS** even helps you to get the peptides spotted on MALDI targets.

◆ **automated in situ detection**

In situ detection of DNA, RNA and proteins are powerful techniques to gain data about the temporal and spatial expression pattern of genes within complete organisms. They can be performed on whole mounts, vibratome sections or thin sections fixed on slides. Our instruments automate most steps of these tedious and labor-intensive methods. The **InsituStain** is our semi-automated solution whereas the **InsituPro VS** has been developed for fully automated high throughput in situ detection on whole mounts, vibratome sections and slides.

◆ **peptide services**

With the **CelluSpots** peptide arrays, protein, receptor and antibody binding studies as well as enzymatic reactions of e.g. kinases can be conducted with a limited number of specimens. **CelluSpots** are peptide arrays, in which hundreds of different peptides are fixed on one slide. The interactions between the molecules to be studied and these peptides can be analyzed on the arrays by means of enzymatic coloring, autoradiography or fluorescence detection.

◆ **challenges of manual procedures**

Although automation is the most sophisticated way to cope with the challenges of tedious and boring routine work in your laboratory, sometimes small, affordable tools already help you to get things done faster and more accurate. INTAVIS offer manual accessories that help to get the work done more efficiently. Chemical reactions on a solid support can be performed much easier and quicker by using our innovative **Disposable Reaction Columns**.

If you want to automate a routine procedure in your lab we are the right partner to help you. With our experience in lab automation and method development we will be able to respond quickly, and find a solution for your specific requirement.

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# 1 Introduction

## 1.1 *InsituPro VS* – field of application

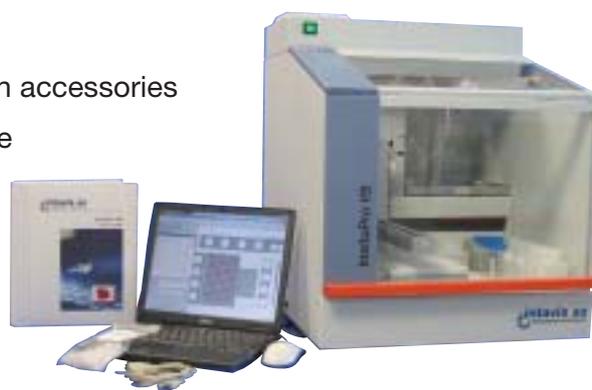
The *InsituPro VS* is a fully automated instrument for in situ detection of DNA, RNA and proteins. It automates various protocols for in situ hybridization, immunocytochemistry and similar serial incubation procedures. The *InsituPro VS* can simultaneously perform in situ detections on up to 96 samples or 60 slides. Typically, the instrument automates all following steps of an in situ hybridization procedure:

- rehydration of specimens/tissue
- permeation with proteinase K
- postfixation and washing steps
- optional bleaching and washing steps
- hybridization with individual probes at elevated temperature
- post hybridization washes
- blocking and antibody incubation
- extensive washing steps
- incubation with AP-buffer

## 1.2 System components

The major components of the system are:

- Safety cabinet with fan
- Pipetting robot
- Dilutor unit with 10 ml syringe
- Work area with racks for 28 different buffers
- Probe rack
- PC to operate the system, with accessories
- Specimen rack or slide module
- *InsituPro VS* User's Guide



### 1.3 Documentation

The **InsituPro VS** User's guide helps the user to reach optimal performance of in situ detection with the **InsituPro VS**. The operator must be familiar with the corresponding manual procedures to use the instrument. This documentation refers to the following **InsituPro VS** system configurations:

- 96 well small basket kit: for small specimens like fly, fish or frog
- 96 well medium basket kit: for medium sized specimens like mouse or chicken
- 48 well large basket kit: for large specimens
- 60 slide kit: for thin sections fixed on standard glass slides

The User's Guide comprises the following sections:

*Quick Guide:*

Short summary of the main operations necessary to carry out an automated in situ detection.

*InsituPro VS Manual:*

Describes the hardware and software of the system as well as operation, maintenance and trouble shooting in detail.

*Method development:*

Gives useful tools that help to develop a method, like loading forms and hard copies of the template protocols. This is the place where you should store hard copies of your favorite method.

*User's Forum:*

Offers all **InsituPro VS** users an opportunity to exchange information. Here you will find information about the latest applications and detailed improvements in method development relevant for your work with the **InsituPro VS**. A more comprehensive and actual user's forum can be found on our website ([www.intavis.com/in\\_situ\\_VS\\_support.html](http://www.intavis.com/in_situ_VS_support.html)).

*Service information:*

Covers all technical information about your **InsituPro VS**, like configuration and calibration data of the system. All service and maintenance reports will be added to this part of the documentation package.

*InsituPro VS User's CD:*

Contains your operation software as well as drivers necessary to establish communication between the PC and the **InsituPro VS**. Moreover, you will find PDFs of all documents of the User's Guide described above.

## 1.4 Structure of the operation software

The system is delivered with a PC dedicated to operate it. The operation software is already preinstalled and tested carefully. Additionally, the system comprises an **InsituPro VS** User's CD including the operation software as part of the User's Guide. The operation software is organized like a file card system holding four different file cards. Each of these file cards features certain functions:

- *Run* 

This menu allows to load, run, pause and stop methods. Beside this you can open a buffer loading sheet as well as log files of protocols used before. You will find a detailed description of all functions in **chapter 5** (Start a method).

- *Edit Method* 

The Method Editor allows to modify an existing method as well as to write a method from the scratch. You will find detailed information about method development in chapter "**Developing a method**".

- *Tray Editor* 

The Tray Editor allows to modify the configuration of the work area. As some parameters defined in this menu are critical for the reliable operation of the system, only advanced users should perform modifications. For detailed information, please have a look at **chapter 7** (Configuration of the work area).

- *Manual* 

This menu gives some helpful maintenance tools for the **InsituPro VS**. Refer to **chapter 8** (Manual mode) for more details.

## 1.5 Customer support

Our product specialists and service staff are available to answer your questions or to assist you in solving problems that may arise. To be able to help you quickly, please provide the following information:

- Type of the instrument, configuration and software version (you can find this information on the configuration sheet in section "Service Information" of this Guide).
- A detailed description of the problem in case of malfunction

To give quick support please send your question by e-mail:

Phone: +49-221-502 94 680

e-mail: [info@intavis.com](mailto:info@intavis.com)

Contact details of your local representative can be found on our web site.



### **IMPORTANT**

***There is also a customer support section on our web site [www.intavis.com](http://www.intavis.com). You will be asked to enter your name and e-mail address before getting access to this area.***



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## 2 Safety

### 2.1 Purpose of use of the *InsituPro VS*

The *InsituPro VS* is designed to automate protocols for in situ detection on whole mounts and sections of a wide variety of specimens.

### **Warning against misuse!**

The *InsituPro VS* complies with current state of the art and safety standards. However, the instrument can pose hazard if it is:

- not used for intended purpose
- operated by a person not especially trained

### 2.2 General safety advice

The instrument shall only be operated by trained and experienced users. The user must know the safety regulations and content of the User's Guide.

- Chemicals must be handled with care. Please observe the current national safety regulations regarding use and disposal of the chemicals used
- Wear safety glasses, gloves and a lab coat during reagent preparation and instrument set-up. If an accidental spill occurs, carefully clean up the spill, taking into account the nature of the spilled component and observe the necessary precautions
- During operation the instrument may generate vapors that pose a potential health risk. For safety reasons keep the door of the safety cabinet closed
- Please make sure that nothing interferes with the movement of the instrument parts. Nothing should be placed in or around the field of movement of either the horizontal or the vertical arm of the robot
- The needle installed on the vertical arm may contain dangerous substances. Do not interfere in the work area of the instrument unless arm and needle have come to a complete stop. Always keep the door of the safety cabinet closed during operation
- The leakage current of this instrument is within the limits allowed by international safety standards for laboratory equipment. An efficient ground connection is imperative for physical protection of the user
- Only personnel trained in such work should perform cleaning, dismantling, maintenance, adjustment and repair
- Voltages present inside the instrument are potentially dangerous. If there is a problem with the instrument, make sure to switch off the unit and disconnect it from the power socket. The power cord should be removed until qualified service personnel have repaired the system
- For safety reasons modifications of the system are not allowed

Genuine parts and consumables were specially designed for the **InsituPro VS**. We have to exclude warranty for damage resulting from:

- Modification of the hardware
- Modification of the software, beyond protocol adaptations within the scope of operation
- Operation deviating from the User's Guide
- Use of non-genuine parts and consumables

### 2.3 Explanations of safety symbols and instructions

The following warnings and safety instructions are used in this **InsituPro VS** Manual:



#### **WARNING**

*This symbol refers to direct hazard to life or health of individuals. Non-compliance with these instructions may result in severe consequences to health or even life-threatening injury.*



#### **CAUTION**

*This symbol refers to potentially dangerous situations. Non-compliance with these instructions may result in slight injuries or damage of the unit.*



#### **NOTE**

*This symbol refers to important advice for a proper handling of the instrument. Non-compliance with these instructions may result in unit malfunction or interference with the environment.*

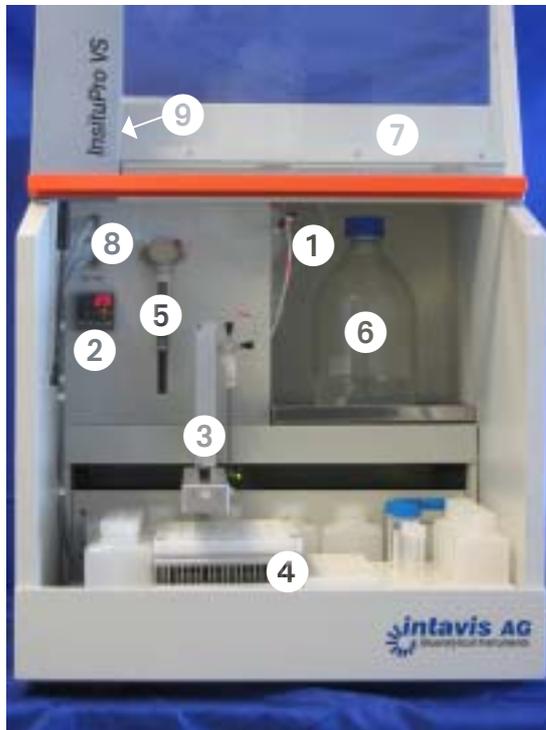


#### **IMPORTANT**

*This symbol refers to useful information about the unit or its applications.*

### 3 System description

#### 3.1 *InsituPro VS* base unit



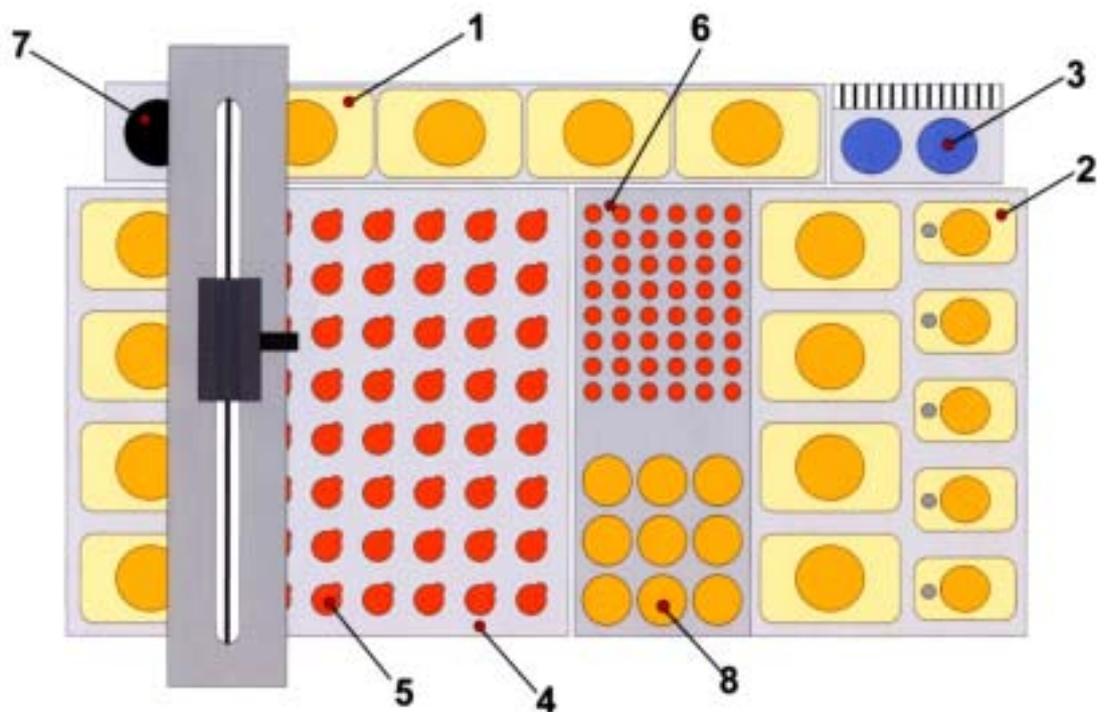
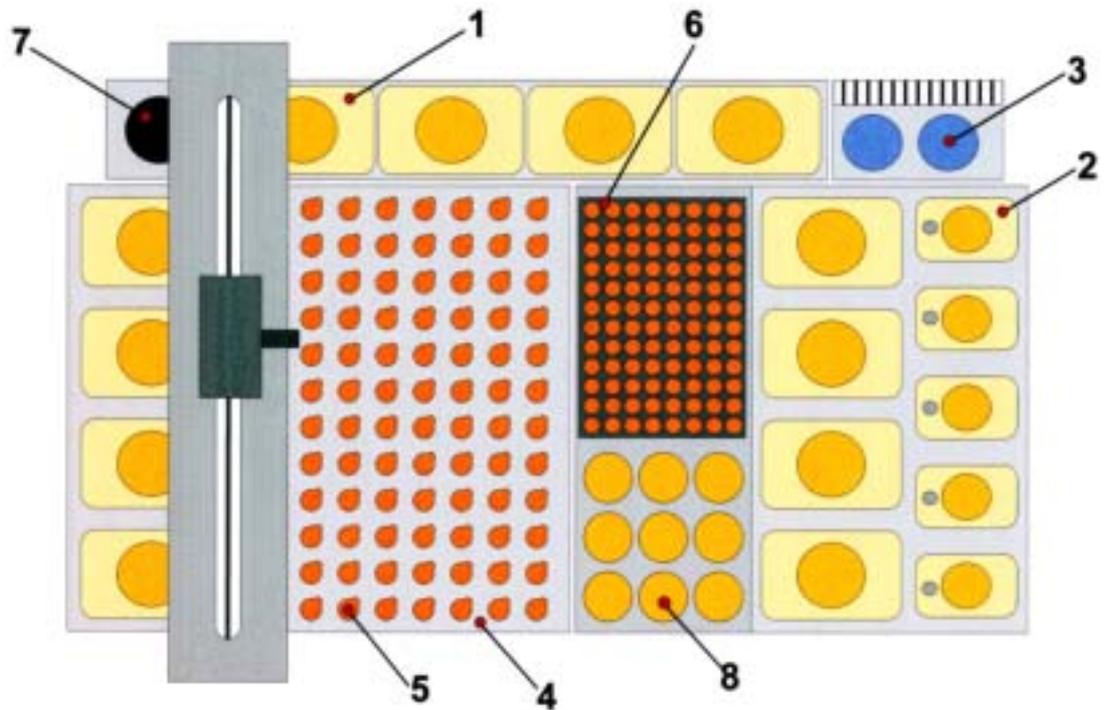
- 1 Transfer tubings
- 2 Temperature controller
- 3 Pipetting robot arm
- 4 Work area
- 5 Dilutor with syringe
- 6 Dilutor reservoir
- 7 Safety cabinet
- 8 Sockets for thermo plate/tub
- 9 ON/OFF switch



- 1 Power socket with fuse holder
- 2 Identification plate
- 3 Connection for PC
- 4 Exhaust fan

## System description

### 3.2 Work areas for baskets



1 Solvent bottle (250 ml)

2 Solvent bottle (125 ml)

3 Buffer vial (50 ml), cooled

4 Specimen tray

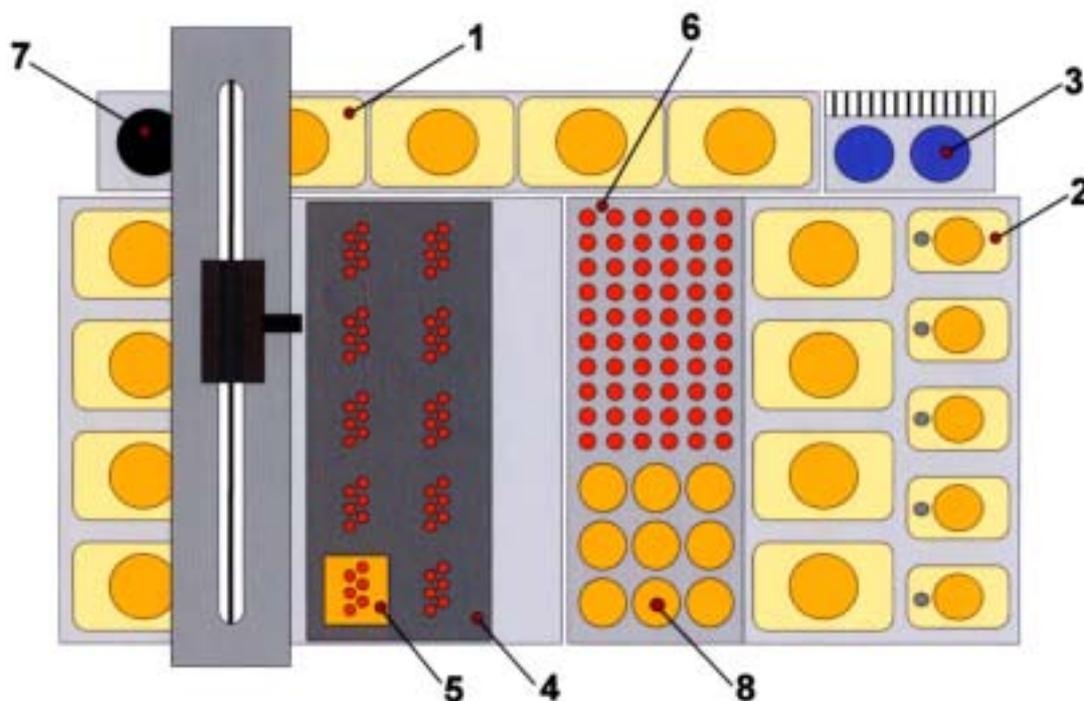
5 Incubation baskets

6 Probe storage

7 Needle rinse port

8 Reagent bottles (14 ml)

### 3.3 Work area for 60 slides



- |   |                         |   |                         |
|---|-------------------------|---|-------------------------|
| 1 | Solvent bottle (250 ml) | 5 | Slide holder (6 slides) |
| 2 | Solvent bottle (125 ml) | 6 | Probe vials             |
| 3 | Buffer vial (50 ml)     | 7 | Rinse port              |
| 4 | Slide tub               | 8 | Reagent bottles (14 ml) |

### 3.4 Components of the operation software

The **InsituPro VS** operation software is preinstalled on the PC that comes with the instrument. You can find the software package in the folder: "Hard disk (C)/INTAVIS/InsituPro VS". This chapter will familiarize you with the basic components of the software. You will find information about the content of the different folders as well as information about the files and the file names used.

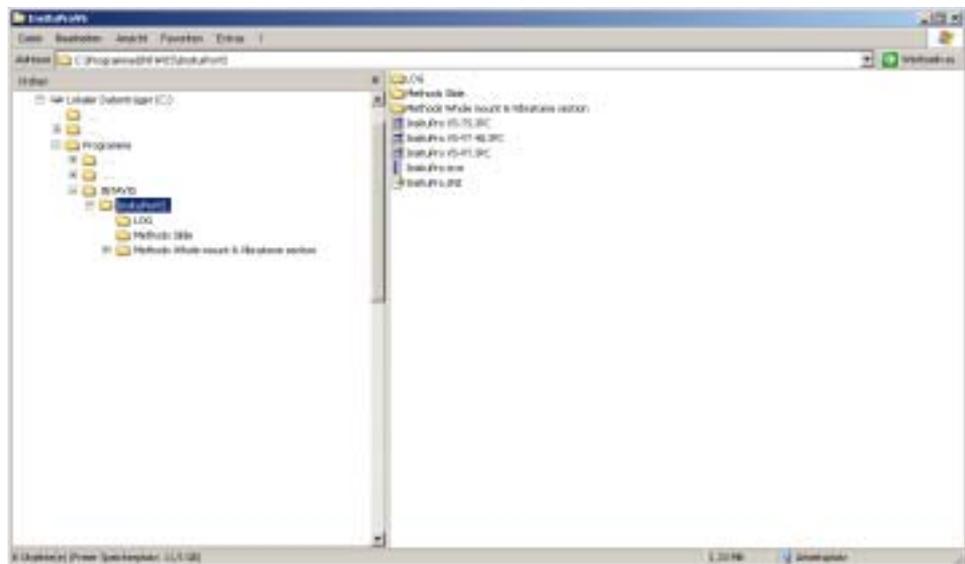
The software folder is structured as shown in the figure on the next page.

- **LOG Folder**  
In the LOG folder you will find log files of each method that have been run on the instrument. Please make sure to clean up this folder from time to time as a huge number of old log files may make it a bit massy.
- **Methods Slide Folder**  
Herein all methods for the slide work area are stored. It already contains the template protocols for slides (see chapter 5.2 for details) as well as a test protocol to check the instrument operation described in chapter 9.5.
- **Methods Whole mount & Vibratome section folder**  
In this folder all methods for the basket work areas are stored. It already contains the template protocols for all basket work areas (see chapter 5.2 for details), a clean method to sterilize baskets prior to their first use (see chapter 9.7) and a test protocol to perform a functional test (see chapter 9.5).

## System description

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- *InsituPro VS.EXE file*  
Click on this executable file to load the operation software. A new one will replace this file if a software upgrade is necessary.
- *InsituPro.INI file*  
The INI file is the memory of the operation software. In this file the operation software stores information about the last method file and the last configuration that has been used, as well as information about the last user.
- *InsituPro VS TS.IPC file*  
This file contains all information about the slide work area, including names and locations of the racks used as well as other information relevant for this work area.
- *InsituPro VS VT.IPC file*  
The *InsituPro VS VT* file contains all information about the 96 well work areas for whole mounts and vibratome sections, including names and locations of the racks used as well as other information relevant for this work area.
- *InsituPro VS VT 48.IPC file*  
This file contains all information about the 48 well work area for whole mounts and vibratome sections, including names and locations of the racks used as well as other information relevant for this work area.
- *All files with the extension IPM*  
These files are method files stored in one of the method folders. The IPM tells the instrument how to process your specimens, tissues or sections.

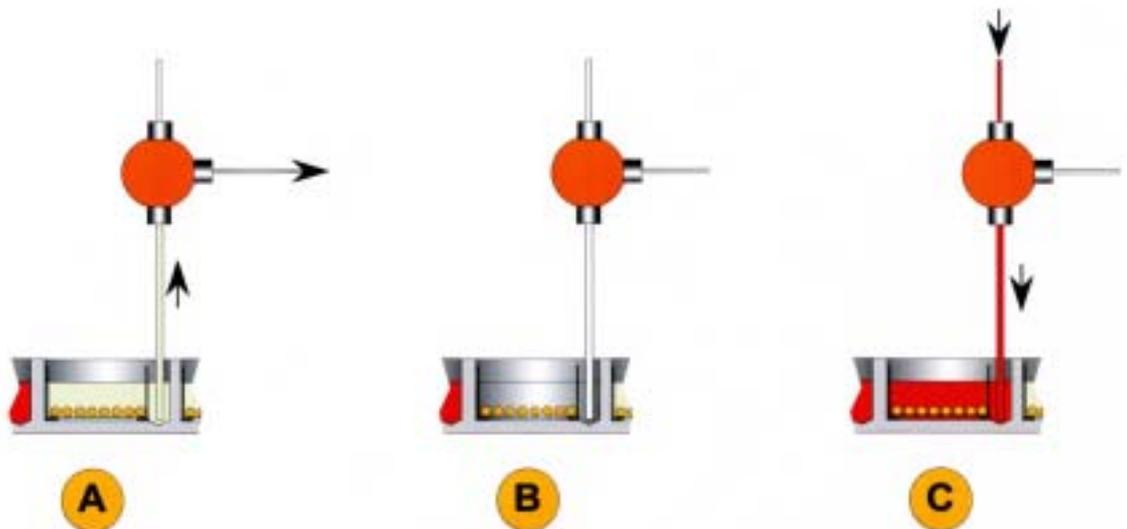


### 3.5 Principle of operation

In situ detection of DNA, RNA and proteins requires incubation of specimens with various reagents and buffer solutions for exactly determined times. *In situ detection* techniques can be divided into two groups: The material is either processed in a floating manner (whole mounts and vibratome sections) or it is fixed to a solid support (thin sections). In most cases glass slides are used. The **InsituPro VS** is able to automate both techniques by using interchangeable configurations.

To process whole specimens or vibratome sections the tissue is put into small incubation baskets closed with a net at the bottom. These baskets are placed in teardrop shaped cavities acting as incubation chambers. A Plexiglas lid covers the whole setup to keep temperature and humidity constant while a method is running. The plate with the teardrop shaped cavities, "Specimen Tray" is placed on top of a thermostated plate, and can be heated or cooled during the process of in situ detection.

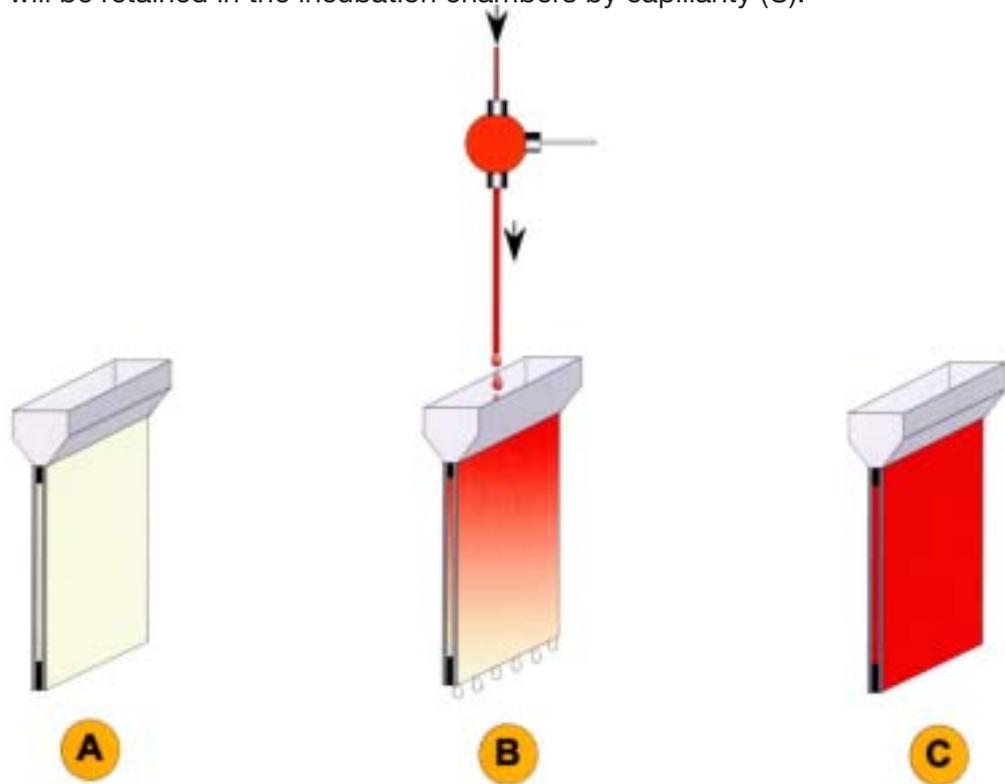
For removal and addition of reagents the pipetting needle is connected to two transfer tubes acting as "Waste" or as "Fresh Buffer" line. Within a liquid exchange cycle the **InsituPro VS** aspirates the used buffer into the transfer tube "Waste Channel" (A). New buffer is added immediately from the second transfer tube (C), the "Fresh Buffer Channel". The time the specimens are not covered with buffer (B) is very short (about a second only). Within this short period of time the incubation cavity acts as a humid chamber keeping the delicate tissue warm and humidified. The entire liquid handling process is optimized for fully automated treatment of even the most delicate specimens. Moreover, it allows recovery of precious probes and solvents.



## System description

Thin sections fixed on glass slides are processed in small incubation chambers made up by the slide carrying the sections and a counter slide with spacers. This slide pair is mounted into a silicon sealing block, that creates a liquid reservoir above the two slides. The sealing block holding the slides is placed into a heatable incubation tub, covered by a lid to create a humid chamber.

For liquid exchange the instrument pipettes fresh buffer into the reservoirs on top of each slide pair (A). This liquid removes the buffer in the incubation chamber by gravity (B). The used buffer just drops into the tub and is flushed into the waste container. When the reservoir is empty, about 150  $\mu\text{l}$  of the added buffer will be retained in the incubation chambers by capillarity (C).



### 3.6 Specifications

The *InsituPro VS* is designed to conform to the following standards:

- EEC directive 89/336/EEC
- EEC directive 73/23/EEC

Specimen size:	0.1 –10 mm and standard glass slides
Work area temperature range:	12 – 70°C
Volume range:	200 –1.000 $\mu\text{l}$
Number of buffer positions:	28 (plus 96 individual probe positions)
Cooled buffer positions:	2
Size of buffer vials:	250, 125, 50, 14 and 12 ml, probe vials from 0.5 to 4 ml
Power:	220/240 V, 50 Hz or 110/115 V, 60 Hz, 250 W
Dimensions:	57cm x 63cm x 69 cm (width x depth x height) (22.4 x 25.2 x 27.6 inches)
Weight:	80 kg

## **4 Installation**

### **4.1 Hardware**

An INTAVIS representative will carry out the installation of the system. Please do not try to install the system yourself. For recommendations concerning transportation of the instrument please contact your local representative.

### **4.2 Software**

Although the system comes with a PC dedicated to operate the system, it might be necessary to reinstall the ***InsituPro VS*** Windows operation software. To do this please proceed as follows:

1. Put the CD labelled with "***InsituPro VS*** User's CD" into the CD drive of your PC
2. Open the folder "Software Setup" and click on the "Setup operation software" icon
3. A setup screen will pop up guiding you through the installation routine
4. Follow the instructions on the screen
5. Restart your PC after you have successfully accomplished the installation

Now an icon schematically showing the ***InsituPro VS*** appears on your PCs' desktop.



## 5 Start a method (RUN)

This chapter describes all steps necessary to run a predefined method on the **InsituPro VS**. To develop or improve a method please refer to chapter “Developing a method” for details.

### 5.1 Preparing the work area

Before starting a run the work area has to be prepared. Please check the points below:

1. Make sure that system and operating PC are switched off
2. Plug the connectors for heating and cooling into the appropriate sockets
3. Load all buffer racks to their appropriate locations (please refer to chapter 3.2 and 3.3)
4. Fill the reservoir bottle with sterile, deionized water
5. Empty the waste container
6. Check all fittings and connections (see chapter 9.3 for details)

### 5.2 Switching on



#### **CAUTION**

##### **Risk of Damage**

***It is necessary to check that nothing hinders the movement of the robot arm or the dilutor syringe. Nothing but the racks and bottles of the work area should be placed in the motion area of the robot arm.***

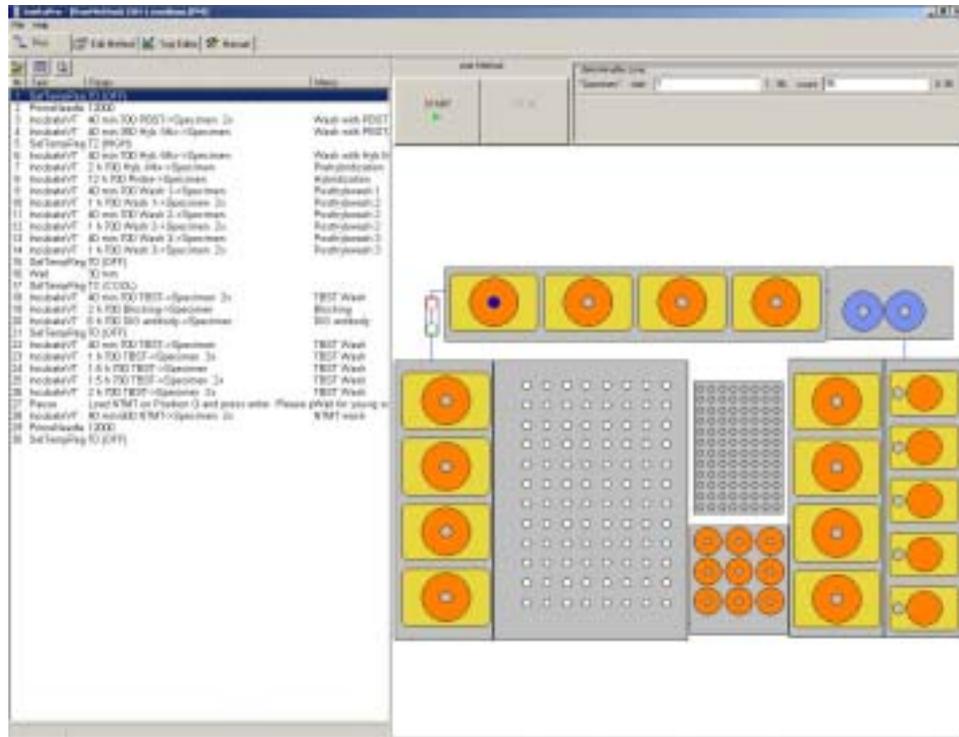
Switch on the **InsituPro VS** by turning the green main switch from “0” to “1”. The switch is now glowing green and the thermo controller shows up with a green and red display. Now start your PC and double click on the **InsituPro VS** operation software icon. The PC starts to communicate with the robot and a screen “User registration” will appear.

- Type in your user ID and the appropriate password
- Select the configuration you like to work with by clicking on the appropriate basket or slide icon
- Confirm your selection by clicking on the OK button



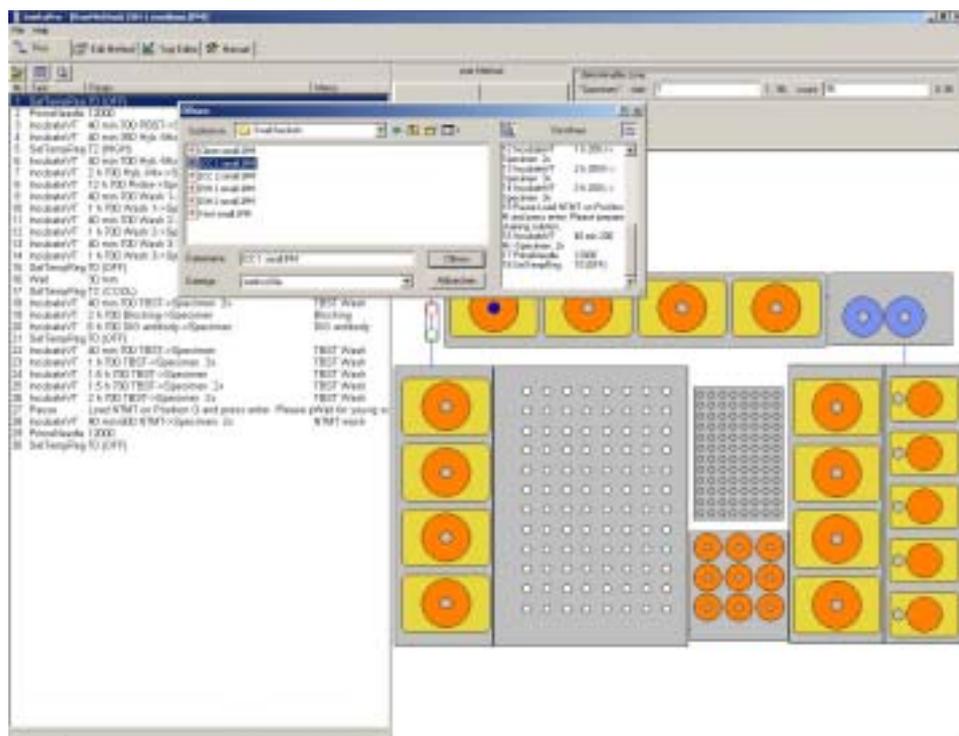
## Start a method

Now the **InsituPro VS** operation software is loaded with the file card “start a run” selected. The last method you have used is activated automatically.



### 5.3 Select a method

To select and load a different method use FILE and open the method you like to work with. Please make sure that the method fits to the configuration selected. Methods for slides are stored in the “Methods Slides” folder whereas the methods for whole mounts and vibratome sections are stored in the “Methods Vibratome & Whole Mount” folder. If the method selected does not fit to the configuration loaded the software will create an error message when you try to start the run.



**WARNING**

*Make sure that configuration and method are compatible. Only use the method that has been developed for a specific configuration.*

**5.4 Volume check function and “Buffer Loading Sheet”**

Before loading buffer, specimens or slides you should calculate the buffer consumption for the experiment by using the volume check function of the software. Please type in the number of samples you like to use for the experiment, go to the FILE folder and activate the „Buffer Loading Sheet“. The software will now calculate the amount of all buffers required and display them in the „Buffer Loading Sheet“.

Well	Buffer	Consumption	Total Volume
A	Sub. H <sub>2</sub> O	1000	1048
B	Wash-1	3000	2078
C	Wash-2	3000	2078
D	Wash-3	3000	2078
E			
F			
G			
H			
I			
J			
K			
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## Start a method

The actual dead volumes are given in the table below.

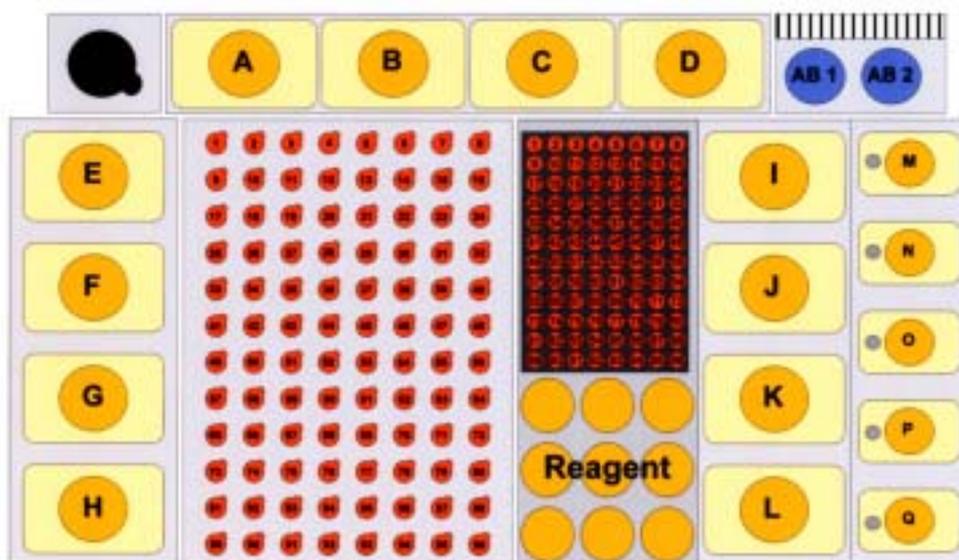
Bottle volume	Position	Dead volume added
250 ml	A - L	15 ml
125 ml	M - Q	10 ml
50 ml	AB 1 and AB 2	1 ml

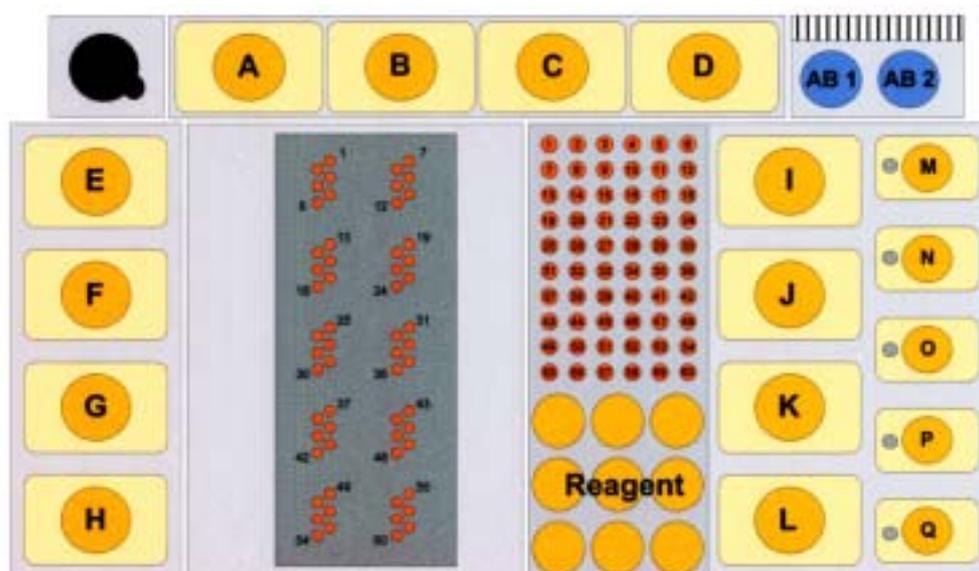
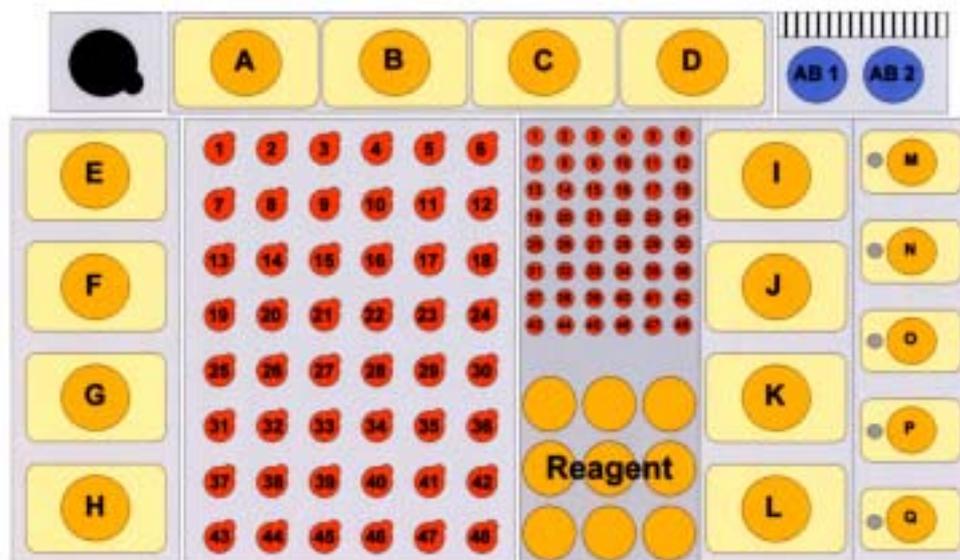
If you use 12 or 50 ml adapters for your experiment, the total volume displayed will be much too high, as it includes the dead volume for a 250 ml bottle. By using the adapters the dead volume for positions A-L can be reduced from 15 ml to 2-3 ml. Please just add 2-3 ml to the volume displayed in the column (Consumption) when loading the 12 or 50 ml vials.

### 5.5 Loading reagents, probes, specimens or slides

#### Loading reagents

Solvents and buffers used in the *InsituPro VS* are prepared according to those used for manual methods. Before each run prepare the buffers as fresh solutions and filter them if they contain insoluble particles. Solvents like 50% methanol or other mixtures of organic solvents and aqueous buffers should be prepared one day before, as these mixtures need some time to degas. Use the buffer calculation feature of the *InsituPro VS* operation software to determine the amounts of buffer required. Fill all buffer vials needed with the amount of buffer calculated and place the buffer into the work area according to the "Buffer loading sheet".





A-L = square bottles, maximum volume 250 ml  
M-Q = square bottles, maximum volume 125 ml  
AB1/AB2 = 50 ml Falcon tubes  
Reagent = 14 ml plastic vials



**IMPORTANT**

*If you use reduction pieces on position A-L please make sure that the amount of buffer (50 ml/12 ml) in buffer vials is sufficient for the method selected.*

## Start a method

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### Loading specimens

The in situ detection is carried out in small, medium or large sized incubation baskets arranged on top of a thermostated plate. Loading the specimens can either be performed with the sample rack mounted on top of the thermo plate (inside the instrument) or with the sample rack removed from the thermo plate (outside the instrument). To load specimens:

- Place incubation baskets into all cavities you will use, observing the numbering scheme
- Fill all cavities that will be used with the appropriate amount of ethanol, methanol or buffer
  - o 200  $\mu$ l for the small baskets
  - o 700  $\mu$ l for the medium sized baskets
  - o 1.600  $\mu$ l for the large baskets
- Place specimens stored in either ethanol, methanol or buffer into the incubation baskets
- Note all specific data of specimens in the loading form
- Place the sample rack with specimens on top of the thermo plate and fix it there
- Close the sample rack with the sealing foil and Plexiglas lid

### Loading the slides

There are two ways to prepare the incubation chambers for the slide work area. You can put the section slide and the counter slide together in a glass beaker filled with sterile water (see pictures below). Alternatively, you can place the counter slides onto a plastic manifold, put 500  $\mu$ l of water on each slide and put the slide with the section onto it (see pictures below). For both ways you have to make sure that the incubation chamber created by the two slides is filled with water and is free of air bubbles.

Mounting slide and coverslide on a plastic manifold:



Mounting slide and coverslide on a glass beaker:



When you finished with a set of six slide pairs please:

- Push the slide pairs into the silicon chambers of the slide holder
- Tighten the four thumb screws of the slide block to fix the incubation chambers
- Load the slide blocks into the slide tray
- Put the slide tray into the incubation tub
- Close the incubation tub with the Plexiglass lid

## Start a method

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### Loading the probes

The probes for in situ hybridization and the primary antibodies for in situ immunocytochemistry are loaded in a deep well microtiter plate or 1.5 ml reaction tubes. Handle the probes with great care to prevent RNase contamination when you transfer them into the appropriate probe container.

- For the slide work area fill the denatured probes into 1.5 ml tubes and put them into the probe rack according to the loading sheet.
- For the basket work area fill the probes into the deep well microtiter plate or 1.5 ml reaction tubes according to the numbering of the specimens in the sample rack.
- Fill each vial with the appropriate amount of probe and note the identity of the probes in the loading form.
  - o 250  $\mu$ l of each probe for the small baskets
  - o 750  $\mu$ l of each probe for the medium sized baskets
  - o 1.600  $\mu$ l of each probe for the large baskets
  - o 250  $\mu$ l of each probe for the slides configuration
- Load the probe rack to its position on the work area.



### **IMPORTANT**

*Make sure that all parts (Specimen tray, incubation tub, lid) are mounted in the right orientation.*

## 5.6 Changing temperature settings

Although the *InsituPro VS* operation software contains a task for changing incubation temperatures called “TempReg” (see chapter 6.5 for details), the temperature setting itself has to be done on the temperature controller directly.

The default temperature settings of the temperature controller are 18°C (Set Point 1) and 60°C (Set Point 2). You can easily change these settings using the soft keys (↺, △ and ▽) of the temperature controller.

When activated the temperature controller is in the standard mode and shows two temperatures.

The upper panel displays the actual temperature of the specimen rack whereas the lower one shows the preset temperature (Set Point 1).

Please follow the instructions below to set the temperatures.

Change lower temperature (Set Point 1).

- Press the ↺ key and SP1 will be displayed in the lower panel of the temperature controller
- Press either △ to increase the temperature setting or ▽ to decrease it
- Press ↺ to confirm the setting

The temperature controller will display SP2 (Set Point 2) now in the lower panel allowing you to set this temperature.

- Again, press either △ to increase the temperature setting or ▽ to decrease it.
- Press ↺ to confirm the setting

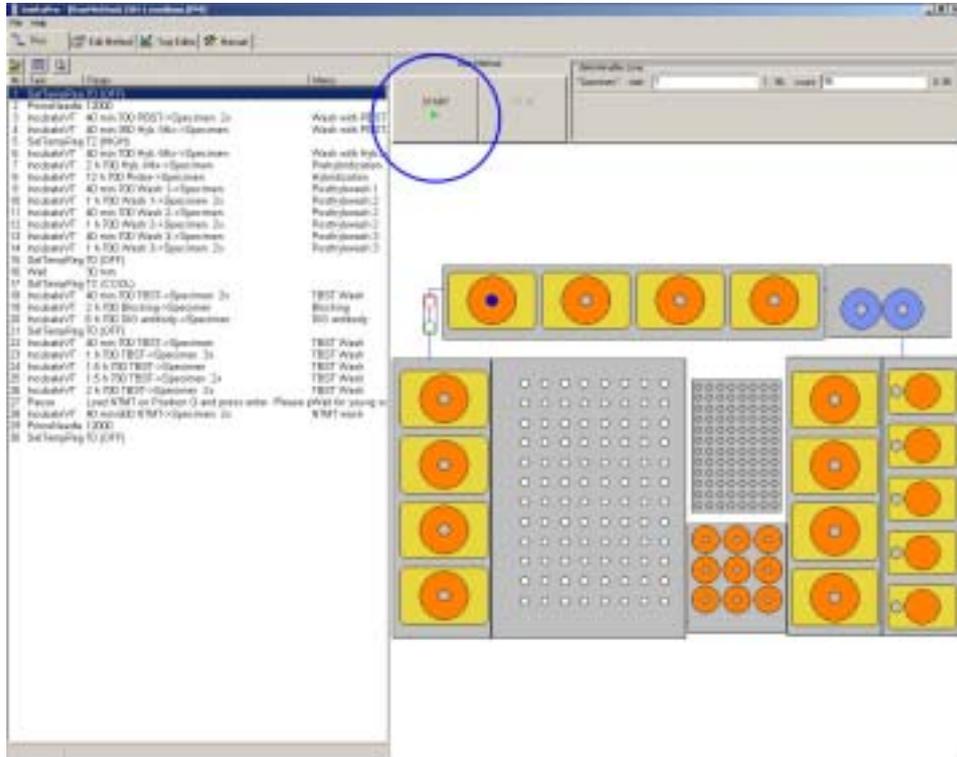
The temperature controller should be back in the standard mode displaying two temperatures now.



## Start a method

### 5.7 Running a method

You can start the **InsituPro VS** after having loaded all buffers, samples and probes. Just click on the Start button (blue circle in figure below).



The **InsituPro VS** will directly start to perform your method with rinsing the dual probe.

### 5.8 Interrupting a run

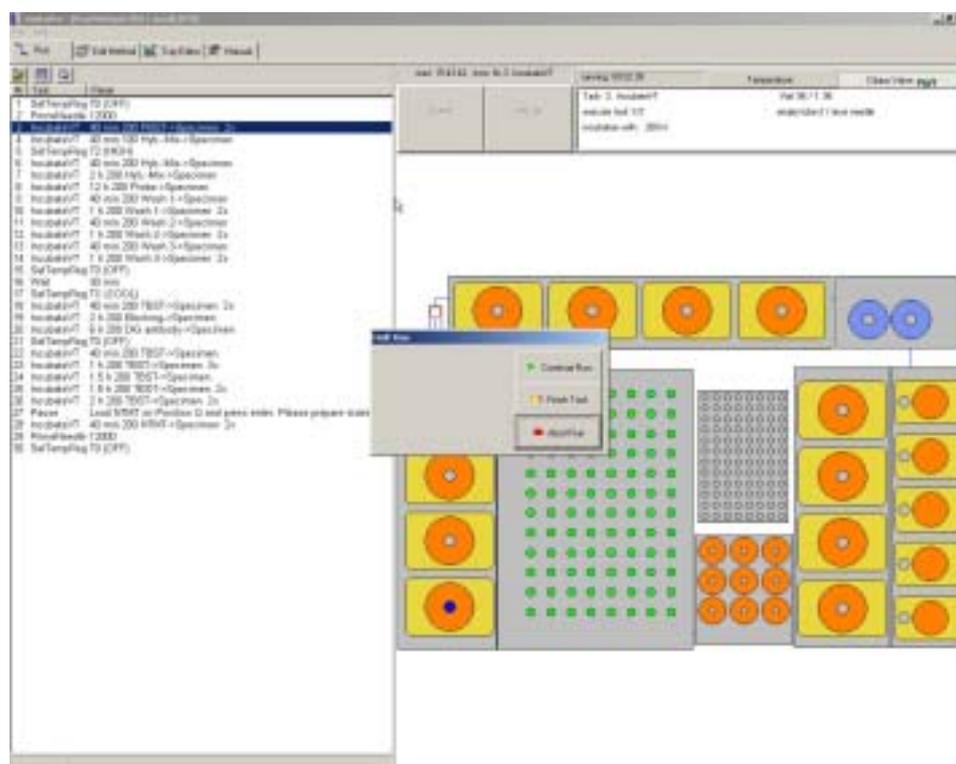
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#### NOTE

**EMERGENCY STOP:** In case of emergency you can switch off the instrument by turning off the main switch while the instrument is working

Interrupting a run might be necessary e.g. to load a forgotten buffer. To pause a run please:

- Click on the PAUSE button highlighted. The **InsituPro VS** will stop working and a message screen appears (see next page).

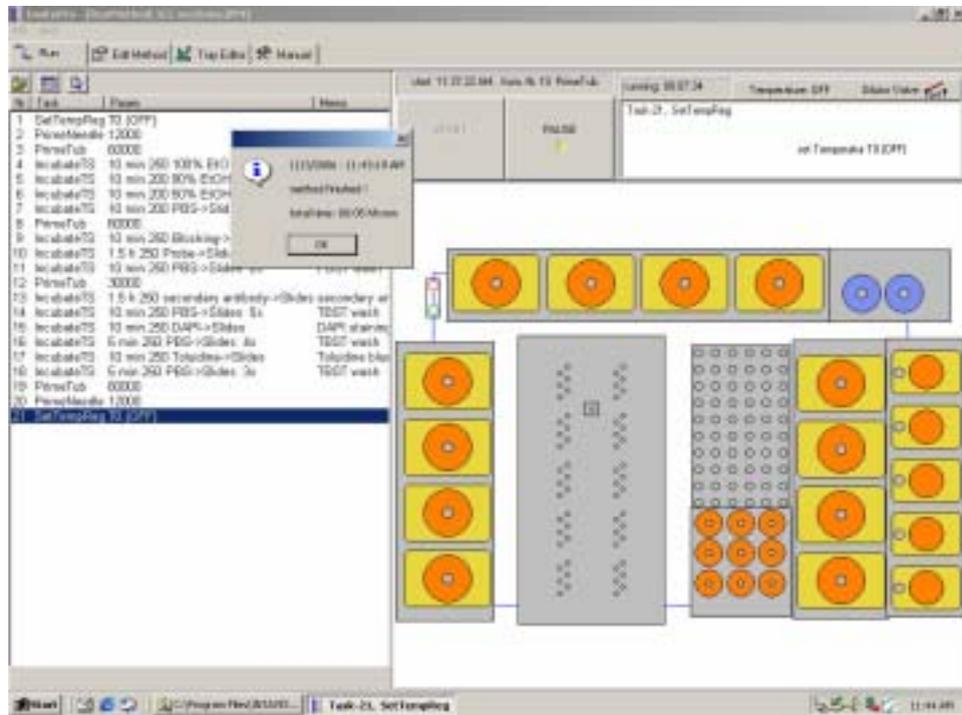


- Select now if you like to:
  - Continue the run:** instrument will resume operation
  - Finish the task:** instrument will finish the task it is working on and will then stop
  - Abort the run:** instrument will abort the run immediately

## Start a method

### 5.9 Finishing a run

After having successfully executed a method the **InsituPro VS** operation software will show up with a window displaying the message



#### **DATE-TIME**

**method finished!**

**total time: hh:mm**

The specimens processed in baskets or the slides are now ready for the final steps (e.g. staining) and should be removed from the instrument.

- close the **InsituPro VS** operation software by selecting the function Exit of the FILE menu
- switch off the **InsituPro VS** using the green main switch

For basket work areas:

- loosen the screws fixing the Plexiglas cover and the ones holding the specimen rack
- remove the complete specimen rack from the thermo plate and proceed as described in the next chapter “Further treatment of the specimens and slides”
- remove the microtiter plates or the vials used for the probes and dispose them according to the safety regulations of your institute

For the slide module:

- loosen the clip fixing the Plexiglas cover of the incubation tub
- remove the slide tray from the tub
- remove the slide blocks from the tray
- loosen the four thumb screws fixing the slide chambers
- remove the counter slides and proceed with the section slides as described in chapter “Further treatment of specimens or slides”

For all configurations:

- remove all buffer vials and dispose the buffers
- empty the waste bottle and dispose its content according to safety regulations
- clean the instrument as described in chapter “Cleaning the instrument”
- remove the vials used for the probes and dispose them according to the safety regulations of your institute
- remove all buffer vials and dispose the buffers
- empty the waste bottle and dispose its content according to safety regulations
- clean the instrument as described in chapter “Cleaning the instrument”

### **5.10 Further treatment of specimens or slides**

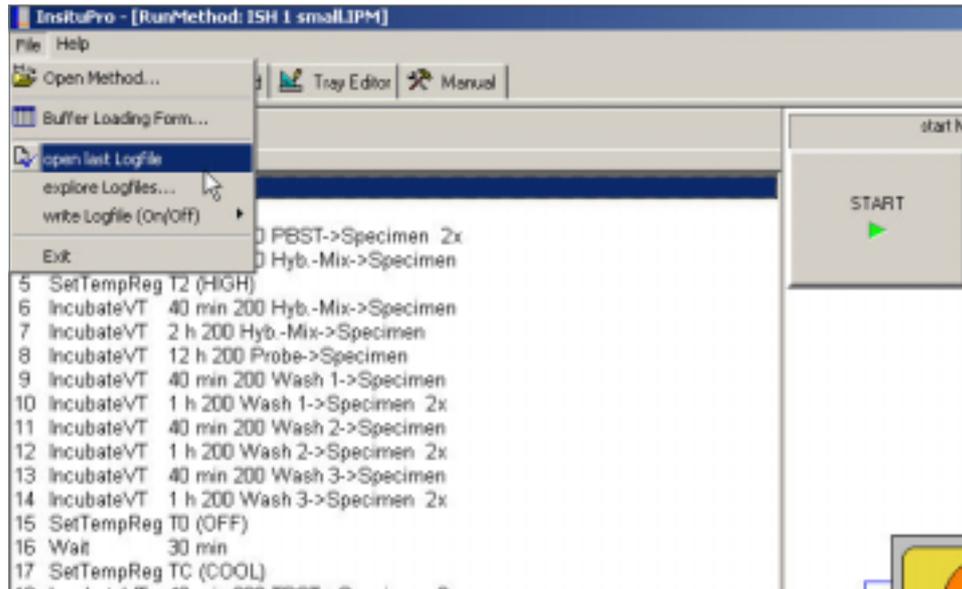
Automated processing of the specimens or slides with the *InsituPro VS* will usually end after unbound anti DIG- or secondary antibody has been removed by extensive washing. The wash buffer has been replaced by a buffer appropriate for alkaline phosphatase or another enzyme used to perform the color detection. The staining itself is usually performed manually. Proceed as follows:

- Transfer the section slides into appropriate containers
- Remove the incubation baskets from the specimen tray
- Transfer baskets into appropriate plastic labware (e.g. tissue culture plates)
- Add fresh staining buffer with substrate (e.g. BM-purple) to start the color development
- Inspect the color development carefully and stop it when the signal has reached a sufficient intensity.

## Start a method

### 5.11 Operation log protocol

Besides the buffer loading form the *InsituPro VS* operation software always creates a Log file (text file) with information about the runs that have been performed on the instrument. You can open the latest Log file by activating the “open last Log file” function within the FILE menu.



A typical Log file (Appendix C) contains:

- The date and time the method has been used
- Information about the software (version no., driver etc.)
- Name of the method
- Complete listing of the method with date and time the tasks have been executed
- Information about the tasks that have been executed

## 6 Developing a Method (Edit Method)

This chapter describes all procedures necessary to develop or adapt methods. The Method Editor makes it easy to optimize your method file and to adapt the template protocols delivered with the instrument. Developing methods from the scratch is also feasible but not recommended.

This chapter also describes the different rights of the administrator and other users and the constituent parts of a method. A detailed description of all tasks a method is made of and a step by step explanation how to adapt manual methods is also included. Finally, you will learn how to save your method and how to simulate a run before using a new method for the first time.

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### **NOTE**

*The InsituPro VS operation software is a powerful and comfortable tool to adapt the instrument to numerous protocols. The flexibility allows you to create protocols specifically fitting to your needs. However, modification of the preset method parameters may ruin your experiment if not performed with utmost care. Please read the following section carefully before carrying out any method modification.*

### 6.1 User levels

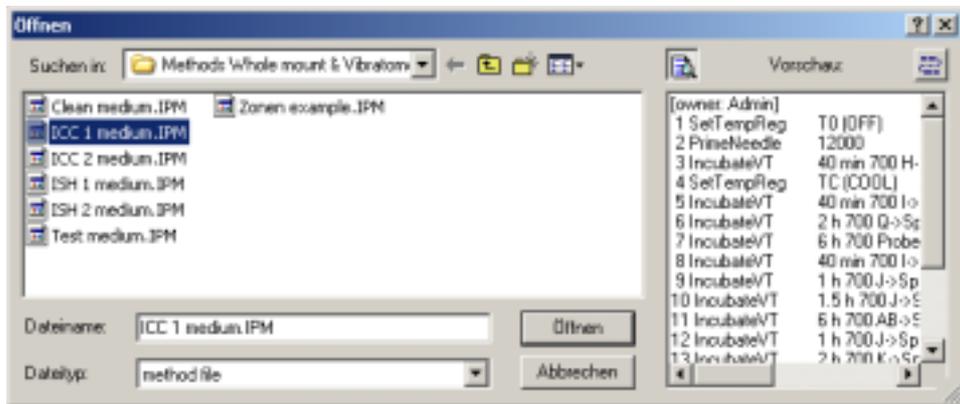
Although the *InsituPro VS* operation software is easy to use, some parts are not accessible to all users. These restrictions effect mostly the configuration of the work area described in chapter 7, but there are also some differences between the Administrator level and the User level in the Method editor:

- The administrator has the right to change and delete all methods, no matter if they derived from his work or from work of other users.
- The administrator has access to all parameters of the tasks described in chapter 6.5
- The administrator has the right to create, modify and delete user profiles
- The user is not able to modify method files owned by other users or the administrator, but he can open these methods to use them directly or as a template for his own method development.
- The user has only restricted access to the parameters of certain tasks

As rights are always accompanied by duties, the administrator has to take special care to keep the system in good shape. The administrator maintenance duties are summarized in Appendix B.

### 6.2 Selecting a template method

It is always easier and much more effective to use one of the existing template methods as a source for further adaptations than to write a new method from scratch. You will find four standard protocols in the Method folders that have been extracted from several protocols published for in situ detections.



There are two protocols, each, for in situ hybridization and for immunocytochemistry. These template method files contain the major steps of in situ hybridization or immunocytochemistry, respectively. The differences are described in the table below:

Protocol	in situ hybridization	immunocytochemistry
Template 1:	Starts with permeated tissue in standard buffer, thus only covering the steps of: prehybridization, hybridization, post hybridization washes, DIG antibody incubation, post antibody washes and ends with the tissue stored in staining buffer.	Starts with permeated tissue in standard buffer, thus only covering the steps of: incubation with primary antibody, post antibody washes, incubation with secondary antibody, post antibody washes. It ends with the specimens in staining buffer.
Template 2:	Includes all steps like: rehydration, permeation with PK, postfixation, prehybridization, hybridization, post hybridization washes, DIG antibody incubation, post antibody washes and ends with the tissue stored in staining buffer.	Includes all steps like: rehydration, permeation with PK, postfixation, incubation with primary antibody, post antibody washes, incubation with secondary antibody, post antibody washes and ends with the tissue stored in staining buffer.

The **InsituPro VS** needs about 40 minutes to perform a complete liquid exchange on all 96 baskets. This is too long for steps like proteinase K treatment that need shorter incubation times. For time critical steps the specimen tray and the slide tub have been divided into smaller subgroups named e.g. "Basket 1-8" or "Slide 1-6". These subgroups allow incubation times of down to one minute. A summary of all predefined subgroups and the minimum time that can be realized by using them is given in Appendix D (see also chapter 7.2).

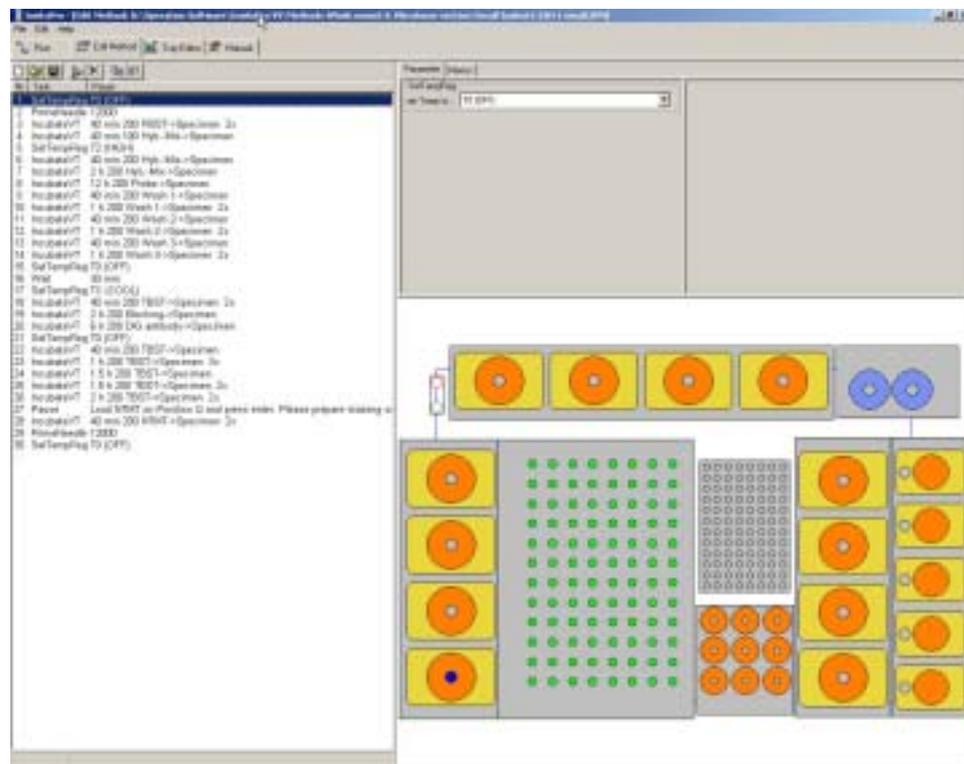
To adapt one of the template methods to your requirements, compare the pre-programmed steps with the content of your manual protocol. If you start with specimens or slides that have been permeated by heat or proteinase K in batches you should use one of the Template 1 protocol for in situ detection. If you like to fully automate your in situ detection select one of the Template 2 protocols.

### 6.3 Constituent parts of a method

A method file consists of several tasks that either transfer liquids, activate the heating/cooling or start other activities of the instrument. Each task is a defined and complete operation. To adapt protocols you only need to choose the tasks you like to modify, set their parameter values to your requirements and arrange them in the method list. All details of the tasks making up a complete method are described below in the chapter “Task description”. During a run, the tasks will be carried out in the order in which they are listed.

### 6.4 Starting the Method Editor

To edit a method, open the *InsituPro VS* operation software and select the file card “Method Editor”. On the left side you can find a complete listing of the method selected, the right side shows a scheme of the work area you are working with.



If you want to change parameters in a task just click on it and adapt parameters shown in the upper right of the display to your specific requirements. A detailed description of the specific parameters available for a task is given in the next chapter. While adapting the task, the scheme of the work area will assist you by e.g. showing source and target zone for the “Incubate” task. When you have finished all your adaptations please do not forget to save the method with a new name.

### 6.5 Task description

A task consists of a series of commands assembled by INTAVIS. It enables you to construct a method for in situ detection. The task controls all of the associated physical functions of your system (moving the arm, switching valves, etc.) As mentioned before all methods for in situ detection used on the **InsituPro VS** are built up by a few tasks only. These tasks are:

<b>Incubate TS</b>	used for liquid handling on the work area for slides
<b>Incubate VT</b>	used for liquid handling on the work area for whole mounts and vibratome sections
<b>Pause</b>	pauses the method until you press enter
<b>Prime Needle</b>	used to prime the liquid handling system
<b>Prime Tub</b>	used to fill and refill the incubation tub of the slide work area with water
<b>SetTempReg</b>	activates the thermo controller and switches from SP1 to SP2
<b>Wait</b>	internal timer

On the next pages you will find a detailed description of the tasks used for in situ detection. These explanations will help you to understand the aim of each task and the parameters available.



#### **IMPORTANT**

*If you are working with the specimen tray or slide tub completely loaded, the minimum time for an incubate step is about 40 min. For incubations shorter than this (e.g. PK treatment) the whole specimen tray or slide tub have to be subdivided in smaller zones. This has been done already for the template protocols. The dependent zones defined allow incubation times down to 1 minute (see Appendix D for details).*

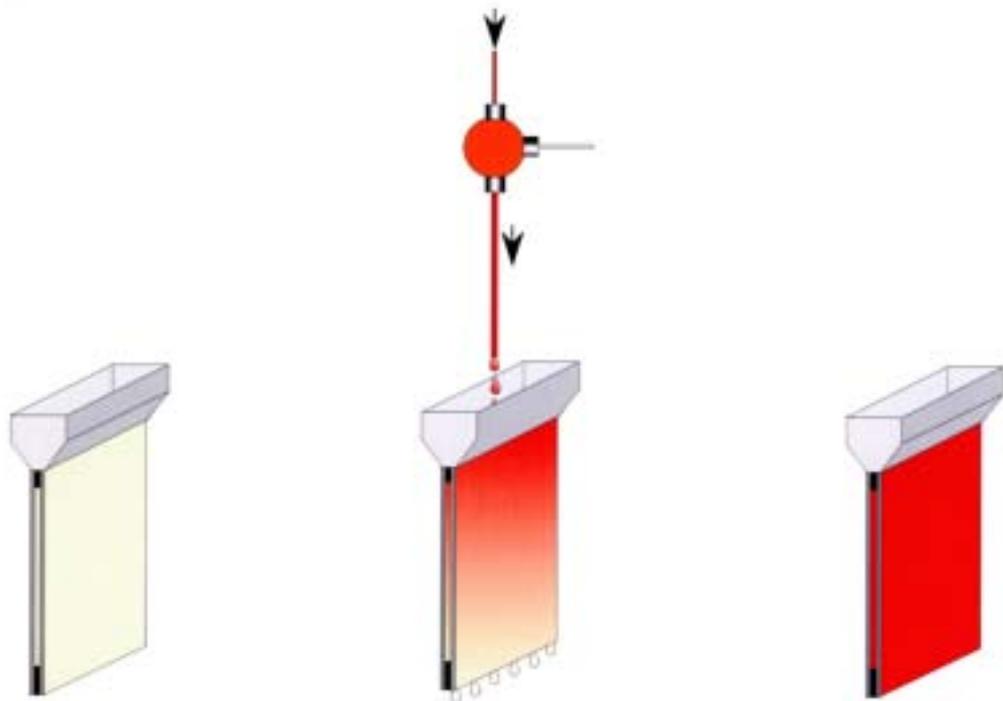
### INCUBATE TS task

This task is used to exchange buffers in the slide chambers of the work area for slides. In contrast to the incubate VT task it does not allow recovery of probes or other expensive solutions. By adding new buffers to the slide chamber the old one is expelled into the thermostated tub, and further into the waste bottle.

Parameter Memo	
<b>IncubateTS/Zones</b>	
sourcezone :	G [PBS]
targetzone :	Slides
execute n :	2 n
incubation time:	
wait time :	0 : 10 h:mm
<b>IncubateTS/Pipetting-1</b>	
dispense volume :	250 µl
aspirate speed :	MEDIUM
dispense speed :	MEDIUM
<b>IncubateTS/Rinse</b>	
inside rinse vol :	NORMAL
outside rinse vol :	NORMAL
rinse dispense sp. :	HIGH

**How it works:**

1. Moves arm to the source zone
2. Aspirates an air gap and picks up the new buffer
3. Moves arm to the first slide selected
4. Dispenses new buffer into the slide chamber, thus replacing the old one
5. Rinses dual probe
6. Waits until the selected incubation time has elapsed



**Parameters**

- |                            |  |
|----------------------------|--|
| <b>source zone:</b>        | Zone where the new solution is aspirated                               |
| <b>target zone:</b>        | Zone into which the solution is dispensed                              |
| <b>execute n*:</b>         | Task is repeated n times   |
| <b>incubation time:</b>    | Duration of the incubation step, selectable units: h, min              |
| <b>exchange vol.:</b>      | Volume of solution dispensed into each slide chamber                   |
| <b>aspirate speed:</b>     | Aspiration flow rate used to pick up buffers from the source zone      |
| <b>dispense speed:</b>     | Dispense flow rate for the delivery of buffers into the slide chambers |
| <b>inside rinse vol.:</b>  | Volume of reservoir liquid used to clean the needle inside             |
| <b>outside rinse vol.:</b> | Volume of reservoir liquid used to clean the needle outside            |
| <b>rinse dispense sp.:</b> | Dispense flow rate used to rinse the needle                            |
| <b>memo:</b>               | Text shown in the list file describing the aim of the task             |

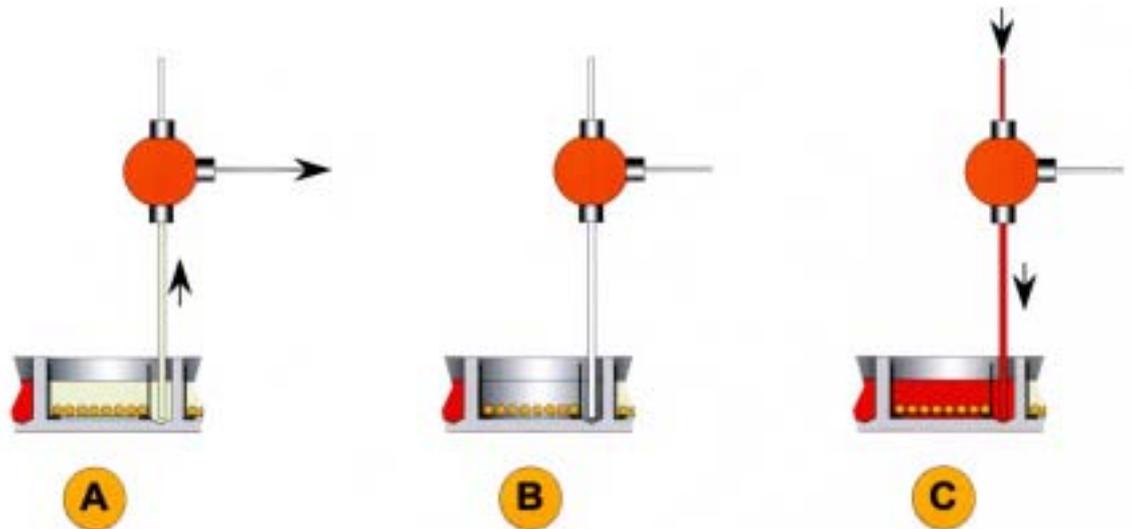
### INCUBATE VT task

This task is used to exchange buffers in the specimen rack cavities of the work areas for whole mounts and vibratome sections. It allows recovery of precious, expensive solutions like the DIG antibody. It also includes an agitation step in which some of the solution is aspirated from the incubation cavity and dispensed back immediately.

Parameter	Value
sourcezone	H (PBST)
recover	<input type="checkbox"/>
targetzone	Specimen
execute n	2
incubation time	
wait time	0 40 min
mix incubation	<input type="checkbox"/>
exchange volume	700 µl
aspirate speed	MEDIUM
dispense speed	MEDIUM
inside rinse vol.	NORMAL
outside rinse vol.	NORMAL
rinse dispense sp.	HIGH

#### How it works:

1. Moves arm to the source zone
2. Aspirates an air gap and picks up the new buffer
3. Moves arm to drain position and adjusts syringe position to zero
4. Moves arm to the first incubation cavity selected
5. Removes old buffer from the incubation cavity and either discards or recovers it:
  - a) if target is a Multiple Use zone (see chapter 7.2 for details):  
Aspirates the preset volume from all incubation cavities and recovers it into the former source zone.
  - b) if target is a Single Use zone (see chapter 7.2 for details):  
Aspirates the preset volume from each single incubation cavity defined and recovers it into the corresponding vial within the single use zone.
6. Dispenses new buffer into the cavities
7. Rinses dual probe
8. Waits until the selected incubation time has passed and mixes the buffer several times, if this feature has been activated.



- A) Used buffer is aspirated from the teardrop shaped incubation cavities into the waste channel.
- B) Incubation cavity is empty for a second but specimens in the baskets are still covered with buffer layer (moist chamber environment).
- C) Fresh buffer is dispensed into the incubation cavity from the second channel immediately.

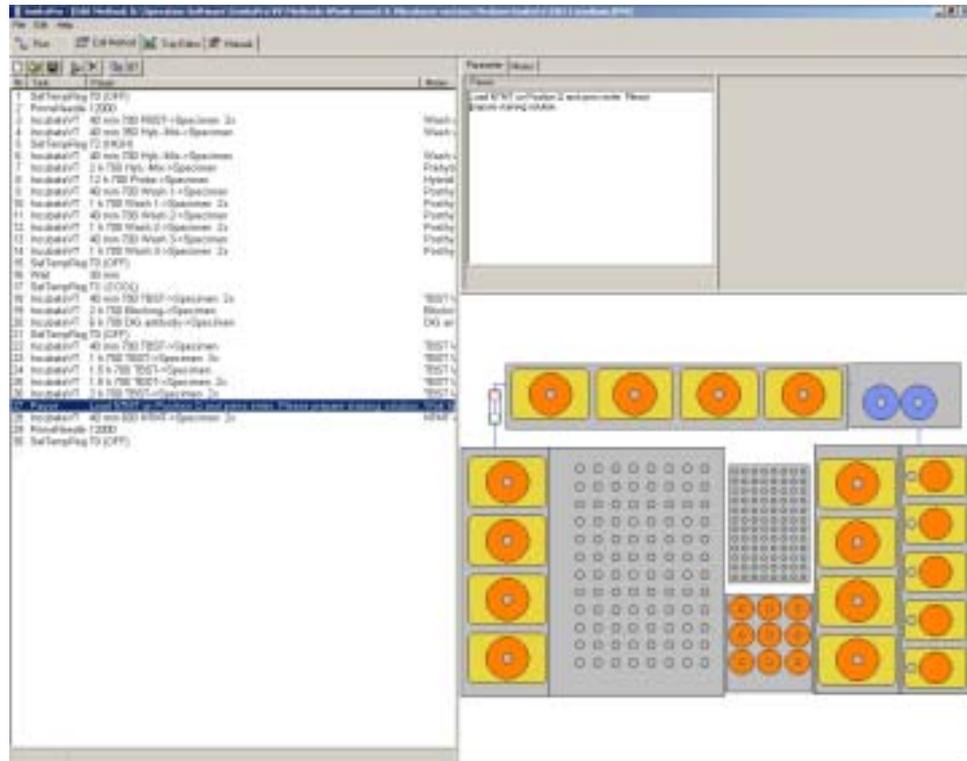
### Parameters

<b>source zone:</b>	Zone where the new solution is aspirated
<b>recover:</b>	If activated, buffer will be recovered into its source vial
<b>target zone:</b>	Zone into which the solution is dispensed
<b>execute n*:</b>	Task is repeated n times
<b>incubation time:</b>	Duration of the incubation step, selectable units: h, min
<b>mix incubation:</b>	Activates agitation by aspirating and dispensing buffer from and into the incubation cavity
<b>exchange volume:</b>	Volume of solution dispensed into each cavity
<b>aspirate speed:</b>	Aspiration flow rate used to pick up buffers from the source zone and to recover the buffers selected
<b>dispense speed:</b>	Dispense flow rate for the delivery of buffers into the incubation cavities or into the buffer vials during recovery
<b>inside rinse vol.:</b>	Volume of reservoir liquid used to rinse the needle inside
<b>outside rinse vol.:</b>	Volume of reservoir liquid used to rinse the needle outside
<b>rinse dispense sp.:</b>	Dispense flow rate used to rinse the needle
<b>memo:</b>	Text shown in the list file describing the aim of the task

## Developing a method

### Pause

This task will pause the execution of a method until you press enter on the PC keyboard. The aim of the Pause task is to allow a safe buffer exchange or to prevent extended storage of the specimens in a non-appropriate buffer (e.g. NTMT).



### How it works:

1. The robot arm is moved to its home position (left side of the work area)
2. A message is displayed on the upper right side of the screen explaining what the user has to do before resuming operation
3. Method is paused until you press "Enter" to continue

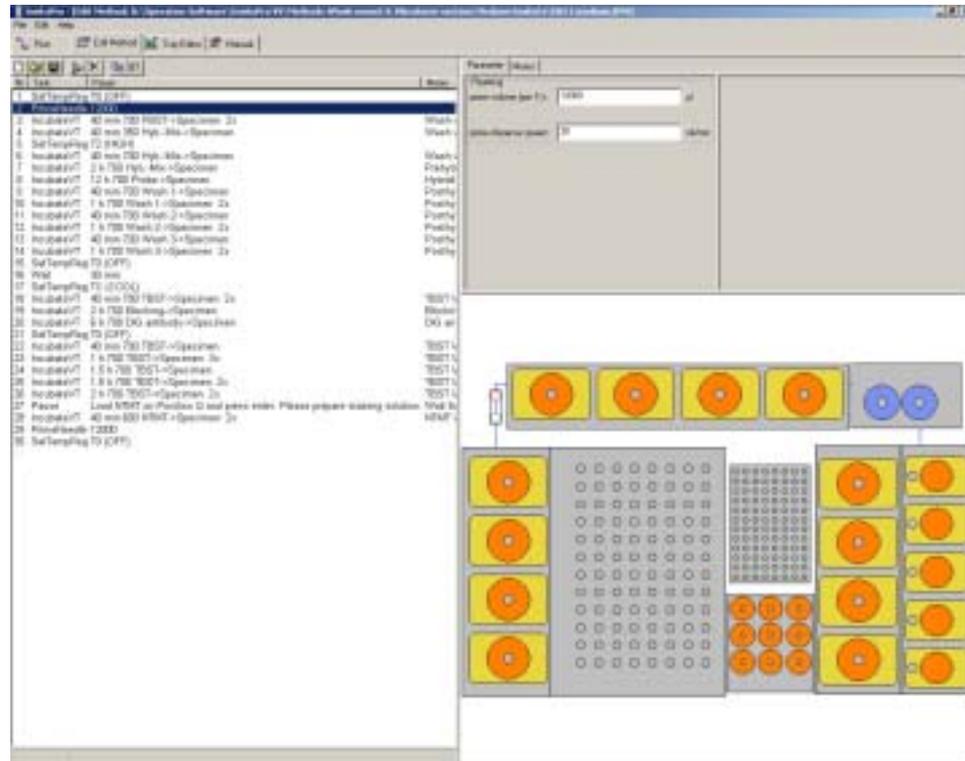
### Parameters:

**text:** The message typed into this field will be displayed when the Pause task is active

**memo:** Text shown in the list file describes the aim of the task

### PrimeNeedle

The PrimeNeedle task is used to clean the pipetting needle intensively and to fill all transfer tubings with reservoir liquid. It is always the first and the last task of the template methods and should also be part of your method.



### How it works:

The robot arm is moved to the rinse/drain port and the preset amount of liquid is used to prime both transfer tubes and the needle.

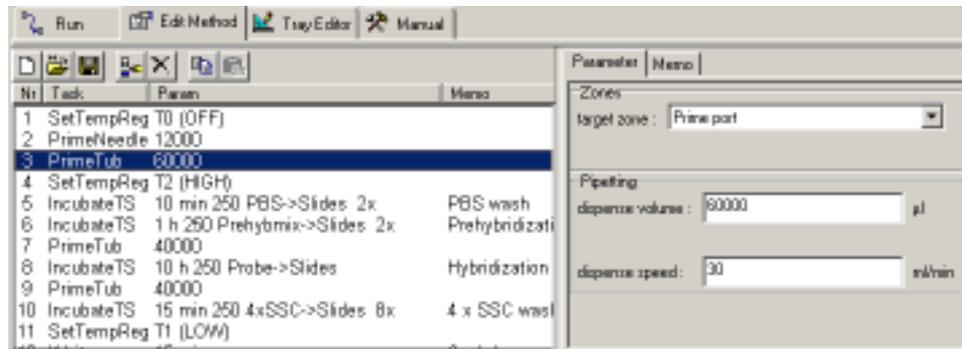
### Parameters:

- prime volume:** Volume in  $\mu\text{l}$  used to prime each transfer tube
- prime dispense speed:** Dispense flow rate used to prime
- memo:** Text shown in the list file describing the aim of the task

## Developing a method

### PrimeTub

The PrimeTub task is used to flush the incubation tub intensively and to fill it fresh with reservoir liquid.



### How it works:

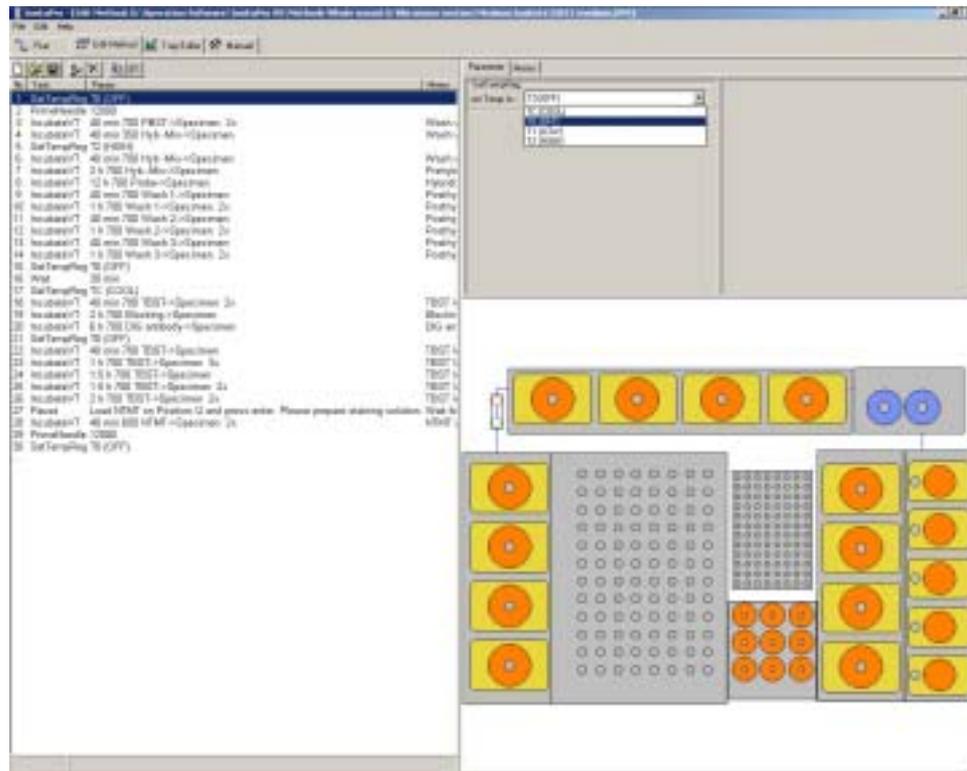
The robot arm is moved to the prime port of the incubation tub and the preset amount of liquid is added into the incubation tub.

### Parameters:

- target zone:** Zone into which the water is dispensed, usually the prime port
- dispense volume:** Volume in µl used to flush the incubation tub
- dispense speed:** Dispense flow rate used to prime
- memo:** Text shown in the list file describing the aim of the task

### SetTempReg

Within a method you may need incubation steps at room temperature, slightly elevated temperature, high temperature or at a temperature below ambient. This task is used to switch between the temperature settings of the thermo controller (see chapter 5.7 for details of the thermo controller) regulating the temperature of the thermo plate below the specimen tray or the slide tub. Although the thermo controller offers two set points (SP1 and SP2) only, the SetTempReg allows to select four different temperatures. Therefore, set point 1 (SP1) can be used for either low heating or cooling depending on the settings of the thermo controller.



**IMPORTANT**

*As the incubation tub of the slide work area is only heatable, the SetTempReg can only be used for heating. The setting TC (COOL) is not working for slides!*

**How it works:**

The SetTempReg either activates or deactivates the thermo controller. It also switches between the two set points of the thermo controller. The temperature selection has to be done on the thermo controller itself. Please refer to chapter 5.7 for further details.

**Parameters:**

**TC (COOL):** Activates Peltier cooling of the thermo plate below the specimen tray. Works only if set point 1 (SP1) on the thermo controller is set to a temperature below ambient. Cools thermo plate to preset temperature

**T0 (OFF):** Deactivates thermo controller. Neither heating coils nor Peltier cooling of the thermo plate or the slide tub is active. Specimen tray or slide tub is at ambient temperature

**T1 (LOW):** Activates heating coils and heats up thermo plate or the slide tub to T1, if set point 1 (SP1) is above ambient temperature

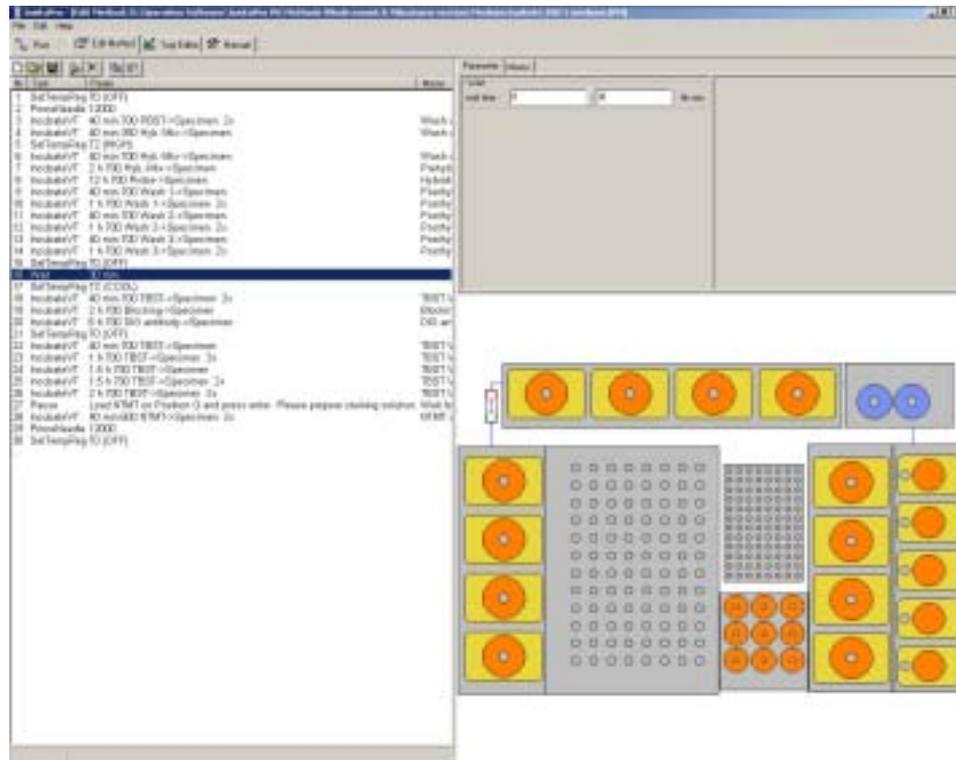
**T2 (HIGH):** Activates heating coils and heats up the thermo plate or the slide tub to T2, if set point 2 (SP2) is above ambient temperature

**memo:** Text shown in the list file describes the aim of the task

## Developing a method

### Wait

This task works like an internal timer. The instrument will wait for the time selected before performing the next task. The “Wait” task is used e.g. to allow the thermo plate or the slide tub to cool down after having operated at elevated temperature.



### How it works:

During a Wait task the *In Situ Pro VS* moves the robot arm to its home position. Once the arm is positioned there, the timer counts down the selected time.

### Parameter:

**wait time:** Enter the time the instrument should wait in hours and minutes

**memo:** Text shown in the list file describes the aim of the task

### 6.6 Editing a method

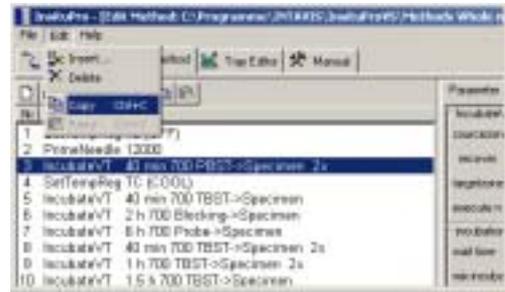
When you operate the *InsituPro VS* for the first time we strongly recommend to adapt your favorite manual protocol using one of the template protocols. This is much easier than writing a method from scratch as most steps are already predefined and you just have to fine-tune some parameters. It is easy to adapt a method if you follow the flow chart below:

- Select one of the template methods (see chapter 6.2)
- Compare your manual method with the template and define start and end of the automated processing
- Get a copy of the buffer loading form of the template method selected (see section “Method Development” of the User’s Guide)
- Adapt the template protocol by adding new predefined tasks, deleting the ones that are not necessary, or modifying the existing ones
- Note the buffer positions within the printed loading form
- When you have finished the adaptation change to the “Run” card and activate the electronic buffer loading form
- Transfer the changes from Buffer Loading Sheet (paper copy) into the electronic Loading Form
- Check the buffer volumes displayed in the Buffer Loading Sheet and make sure that none of them exceeds the bottle volume (will be displayed in red)
- Close the buffer loading sheet and check the method listing again for consistency
- Save the method with a new name (Chapter 6.11)
- Simulate a run to check all settings (Chapter 6.13)

### 6.7 Copy/Paste a task

There are three ways to copy and paste a task or a group of tasks. You can either use the “Edit” menu, the copy and paste icons displayed in the tool bar, or a simple click on the right mouse button on the highlighted task. Just use the one that is most comfortable for you, the procedure is always the same:

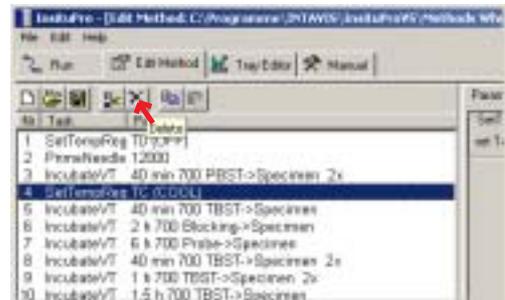
- Highlight the task or the group of tasks you like to copy and click on “Copy”
- Select the task in the method listing above the position where the selected task should be pasted, and press “Paste”



### 6.8 Delete a task

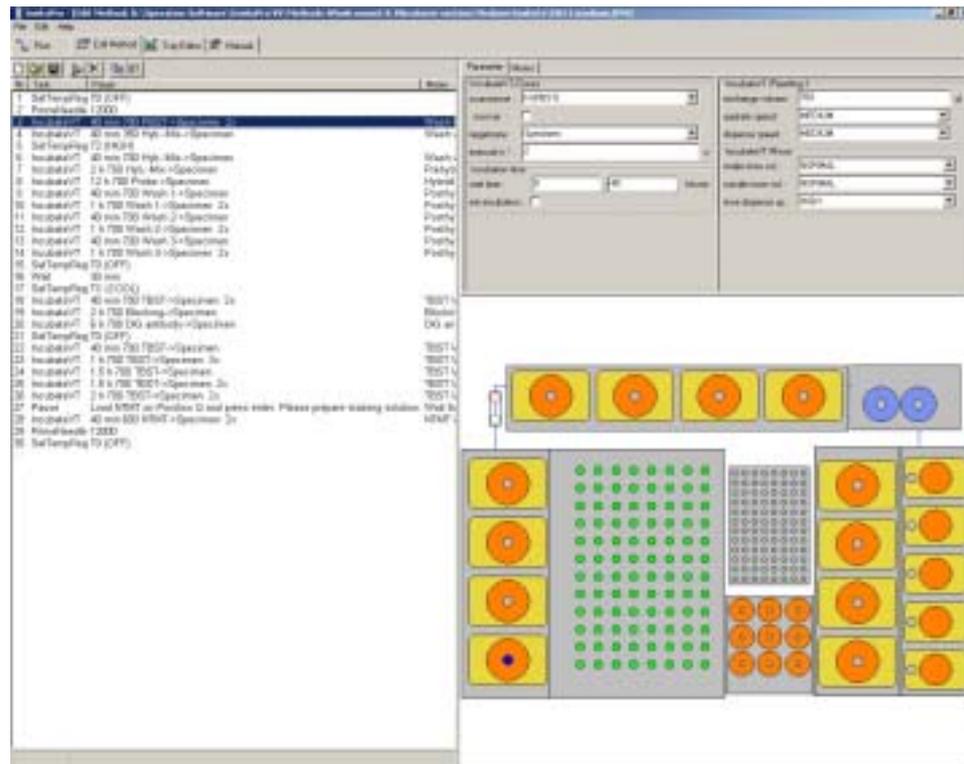
Like for the “Copy/Paste” function you can choose between three ways to delete a task. Use the “Delete” function of the Edit menu, use the delete icon in the tool bar or activate the delete function with the right mouse button. To delete a task:

- select the task you like to erase and delete it



### 6.9 Modify a task

To modify a task just select the task you like to work on and change parameters such as incubation time, pipetting speed etc. according to your requirements. A detailed description of all parameters is listed above in chapter 6.5. Please keep in mind that not all parameters are accessible for a normal users, as some are classified as critical. Changing these parameters either influences the reliability of the operation or may even harm parts of the *InsituPro VS*.



### 6.10 Insert a new task

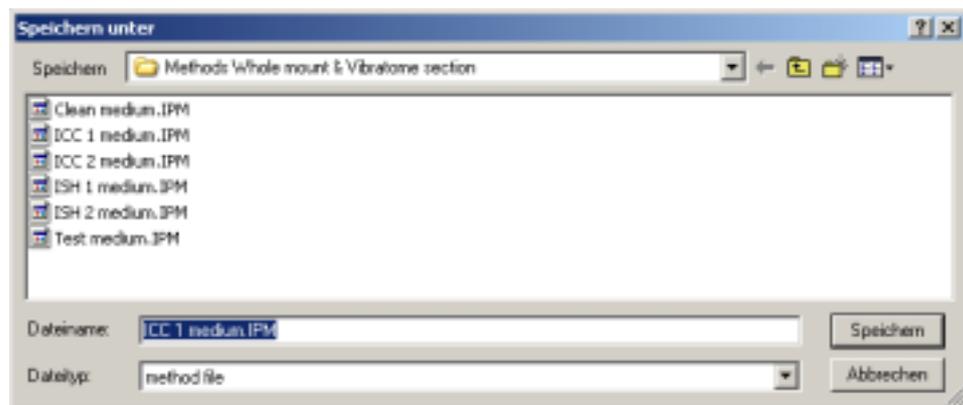
Once again you have a choice of three ways to insert a task. Use the insert function of the “Edit menu”, use the insert icon in the tool bar or activate the insert function with the right mouse button. The new task will always appear above the one that is selected. To insert a task:

- select the task below the position where the new task should be put
- Click on “insert”
- Choose a task from the task list that appears
- Edit all parameters



### 6.11 Saving a method

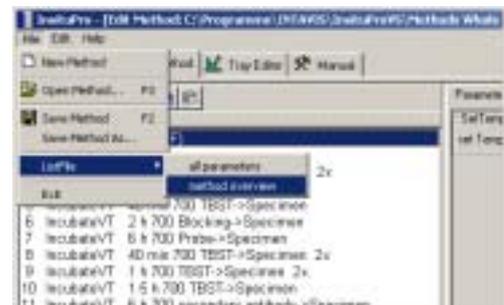
There are two ways to save a method after you have finished all adaptations. You can either select the “File menu” and save the method with the given name or save it with a different name by using the “Save method as” function. The template protocols are all protected against modifications. You can only save a modified template protocol with a new name using the “Save as” function. The same is true if you select the protocol of another user protocol as a template. The second way to save your modified method file is to just click on the “save method” icon in the tool bar.



### 6.12 Creating a method List file

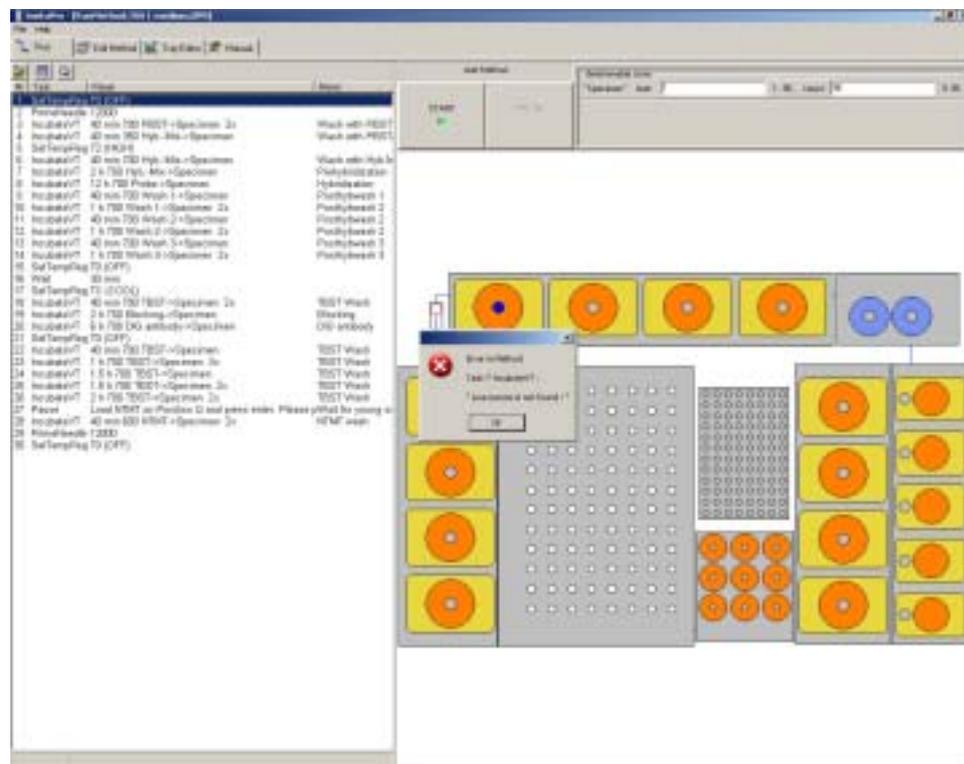
The function “ListFile” in the menu File allows to create a complete listing of your method. This list file is a text file that can be opened by any text processing program (like Word etc). The list file is a copy of your method and contains information about the configuration of your instrument. You have two options to create a list file. You can either select “all parameters” or “method overview”. The option “all parameters” creates a listing that includes parameters of all tasks the method contains. Such a file can be remarkably long (up to 40 pages). The “method overview” just displays a listing of the method comparable to the one you can see on the screen, thus the text file created is quite short (2-4 pages). All list files created are saved automatically in the folder the related method is stored.

The “ListFile” function is very useful to report application problems or to exchange protocols with other users.



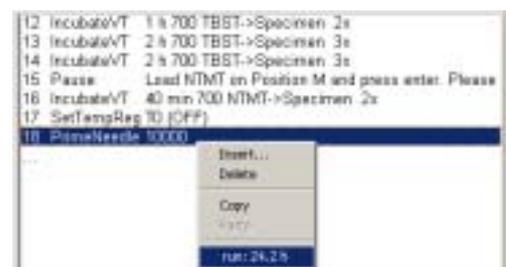
### 6.13 Simulating a run

Before you use a new method or the modified method with real specimens we recommend to simulate a run. This simulation will pinpoint any mistake within your protocol like zones that are not defined etc. To start a simulation just activate the **InsituPro VS** operation software without switching on the instrument. The software will recognize that there is no robot connected and ask to go into virtual mode. Select this and continue with booting the software. Once the software has been loaded just load your method and press “Start”. If the method is compatible to the predefined configuration, the simulation will start immediately. If not, a failure message will come up stating e.g. that a target zone is not defined. Please correct the target zone for the task causing the error message, and try the simulation again.



### 6.14 Estimating the duration of a method

The **InsituPro VS** software allows to roughly estimate the duration of a method at each step. To activate this function just select a task and click on the right mouse button. Within the last line of the window that appears a time is displayed. This is the time span from the method start to the beginning of the task you selected. The pure liquid handling time is not added. As there are long incubation times within the methods (hybridization etc.) the calculated time is quite close to the exact duration of a method.





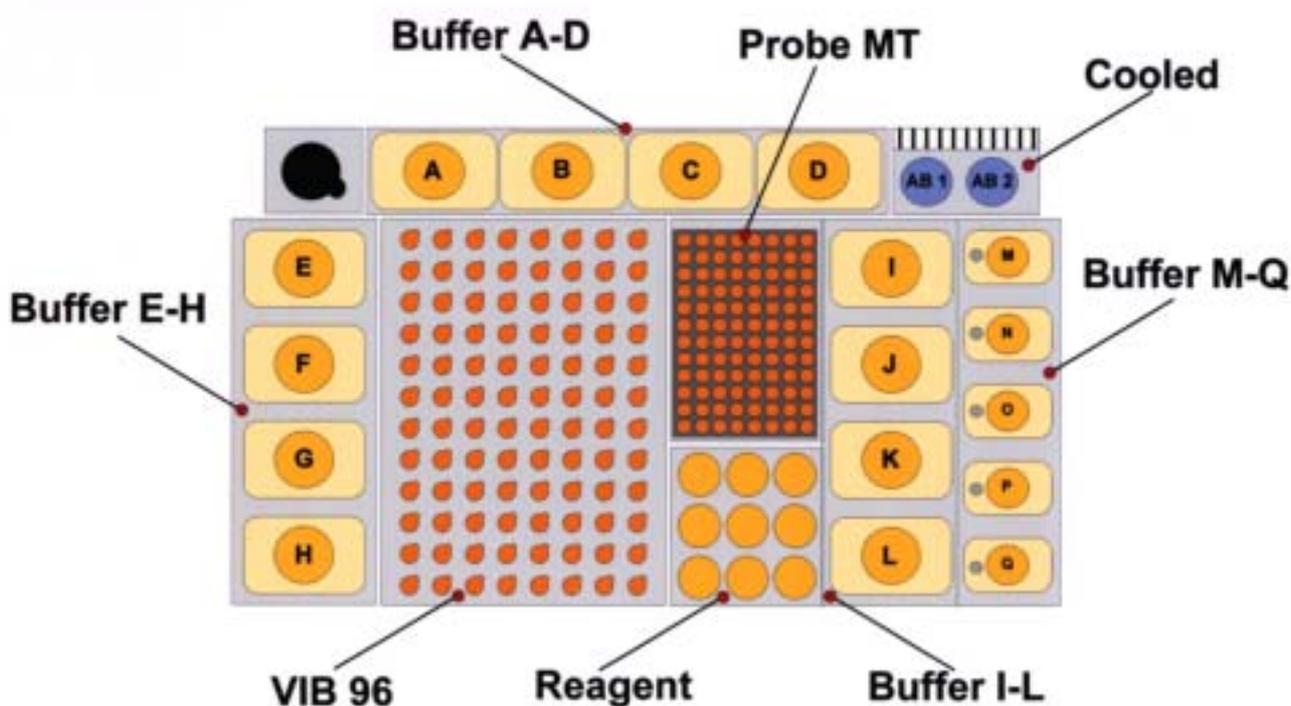
## 7 Configuration of the work area (Tray Editor)

In this chapter you will find information about the work area configurations available for the *InsituPro VS*. You will learn how to calibrate rack coordinates and how to modify or create zones. The system administrator only has full access to all configuration parameters, as modifications may lead to destruction of system components.

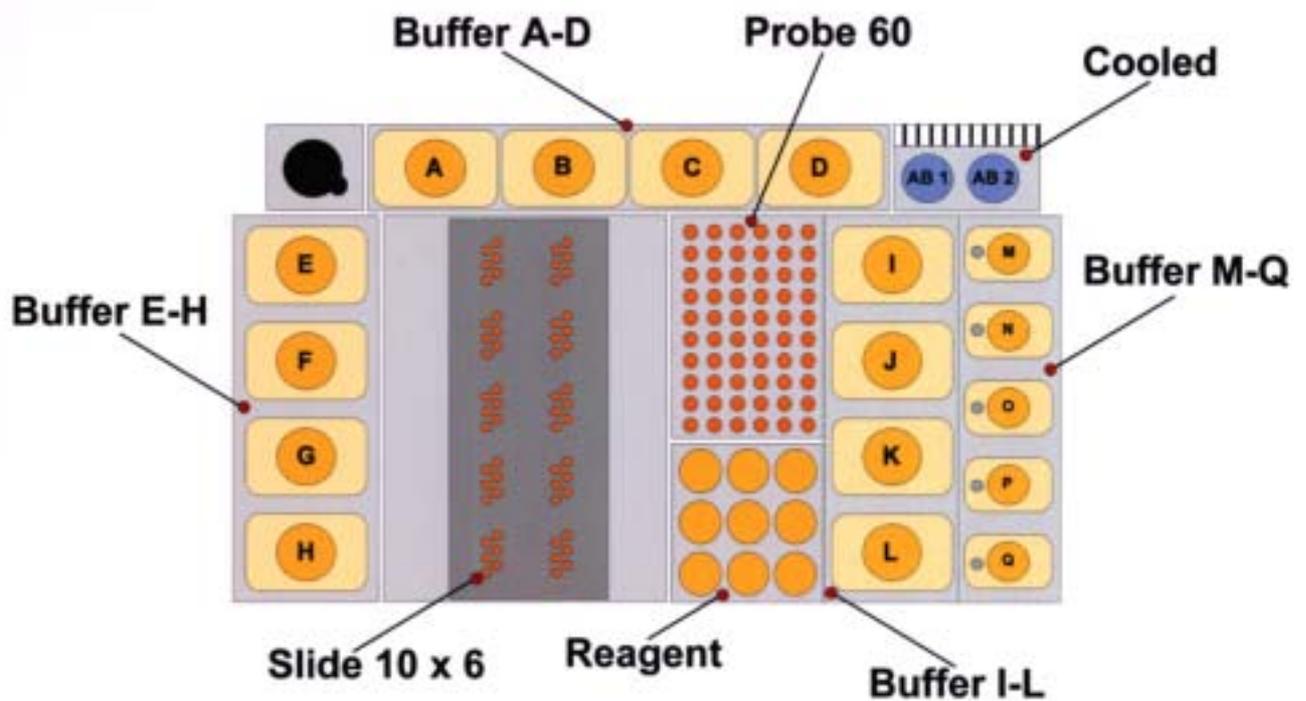
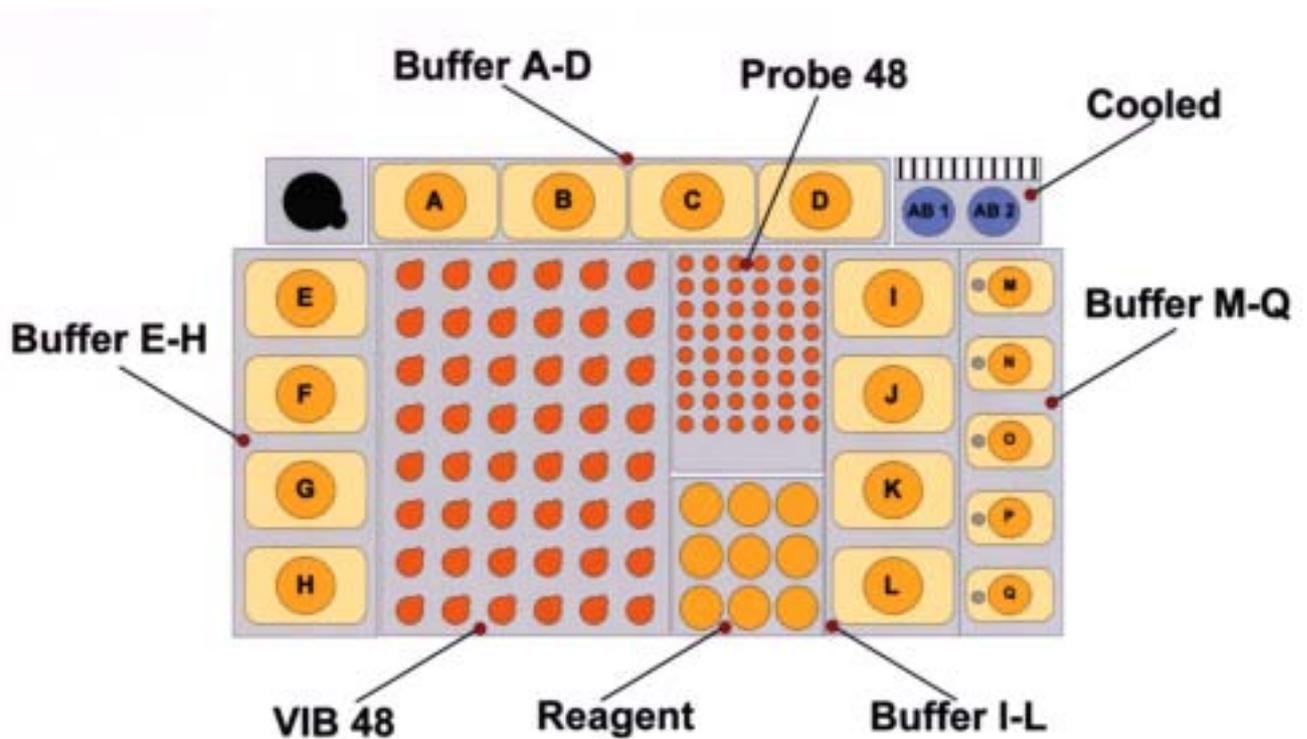
The “Tray Editor” menu is subdivided in three file cards named racks, zones and vials. Each of them offers different features described below.

### 7.1 What is a configuration?

A configuration describes the physical racks, the zones defined and the vials used to build the work area. As the *InsituPro VS* is available with four different work area configurations (three for whole mounts and vibratome sections and one for slides) you will find three predefined basic configurations.



## Configuration of the work area

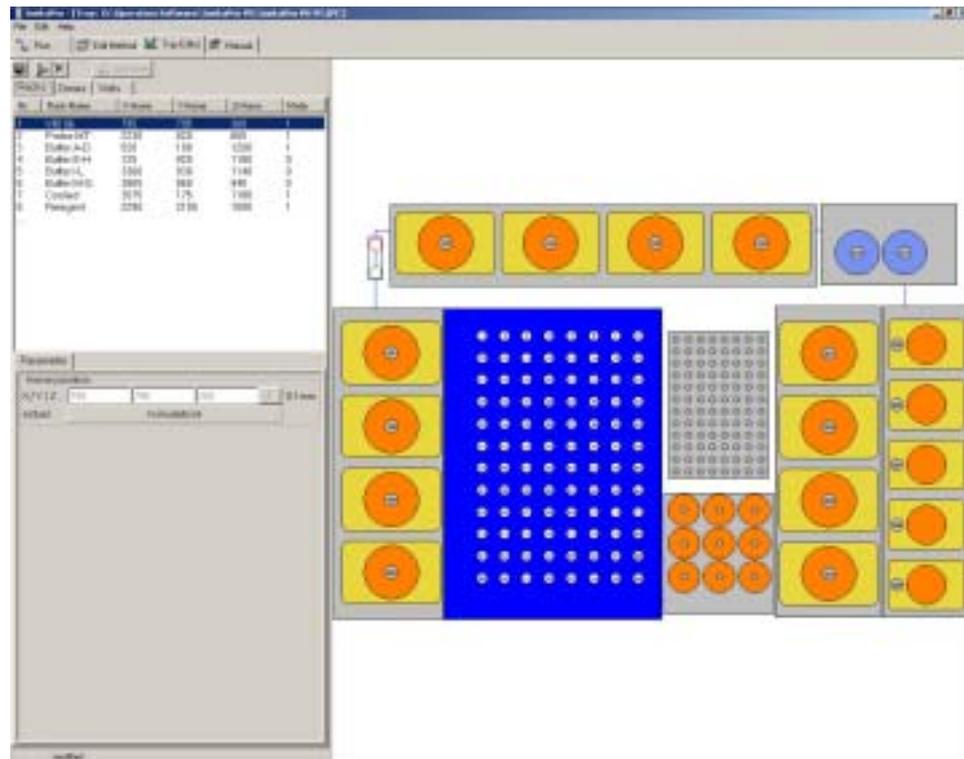


### CAUTION

The InsituPro VS is configured by INTAVIS and will be adjusted to the work area supplied during installation. Any modification of these files may harm the instrument and should only be performed by advanced users.

## 7.2 Tray, rack, zone and vial definition

The work area (Tray) holds racks, containing vials, which are grouped in zones. Racks are physical entities that can be moved around or taken out. A rack has a rack home coordinate in the XYZ system of the robot, which defines all other vial positions. This rack home position is always defined on XYZ coordinates of the rear left vial. The values displayed in the table of the rack file card are given in 0.1 mm units relative to the rear left corner of the work area.



For the **InsituPro VS** work area thirteen different racks are predefined (see fig. above and Appendix E):

- VIB 96: Holding small and medium baskets of the whole mount and vibratome work area
- VIB 48: Holding large incubation baskets of the whole mount and vibratome work area
- Slide 10x6: Holding the slide chambers of the slide work area
- Probe MT: Holding the microtiter plate for probes in the small and medium basket work area
- Probe 48: Holding the 1.5 ml tubes for the probes in the large basket work area
- Probe 60: Holding the 1.5 ml tubes for the probes in the slide work area
- Prime port: Port used to flush the incubation tub
- Buffer A-D: Holding buffer bottles A-D
- Buffer E-H: Holding buffer bottles E-H
- Buffer I-L: Holding buffer bottles I-L
- Buffer M-Q: Holding buffer bottles M-Q
- Reagent: Holding nine additional vials of 14 ml, each
- Cooled: Holding two cooled 50 ml buffer vials

## Configuration of the work area

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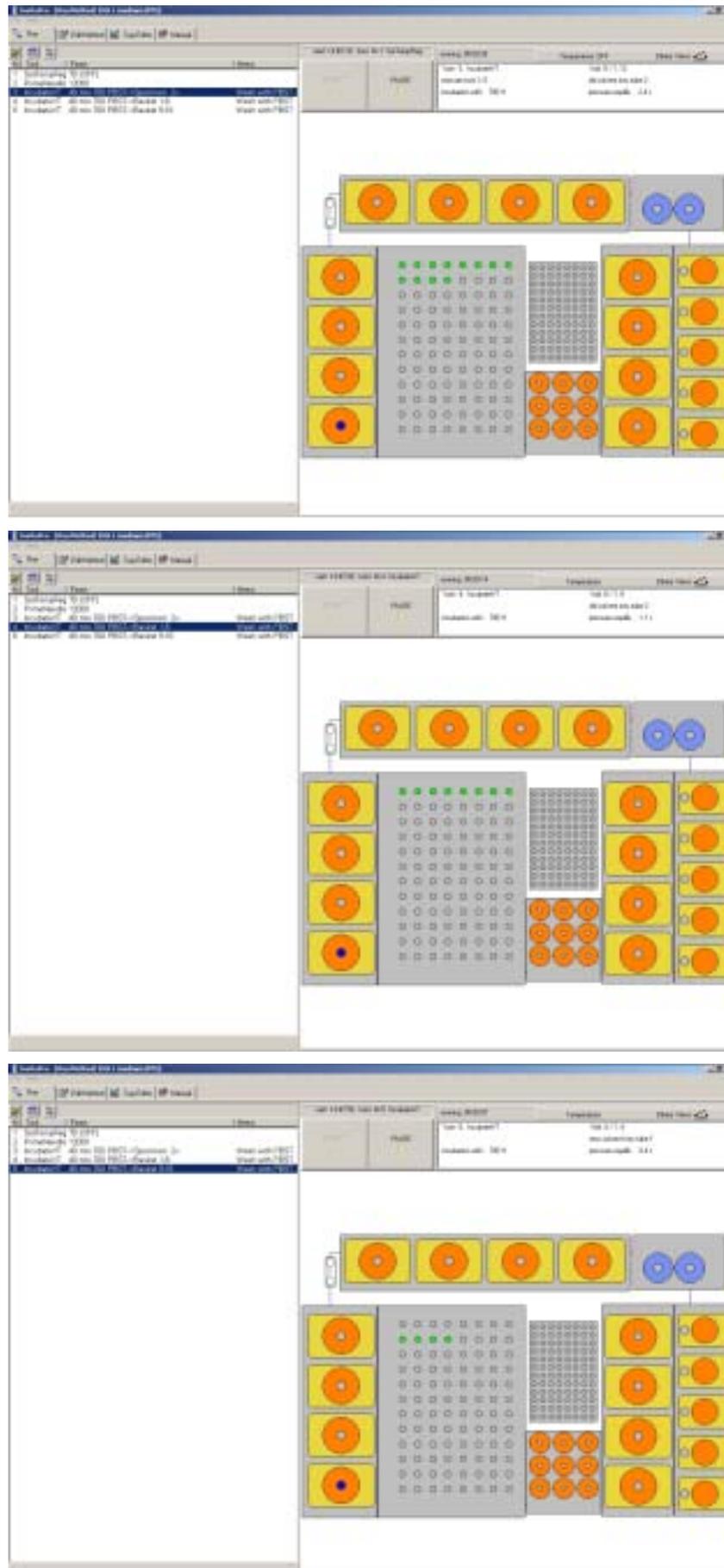
Some of the predefined racks like e.g. Buffer A-D are used in each configuration whereas others are only used for one configuration. Therefore, the total number and names of the racks displayed differs depending on the configuration loaded (see Appendix E for details).

The most important concept is grouping of vials to “Zones”. A zone signifies a label on a group of vials, identifying them e.g. as specimens or buffers. The software will address vials only by the zone name assigned to them. A zone can be a full rack, part of a rack, or an individual vial. The number of active vials in a zone can be fixed (e.g. for all buffer vials) or variable (e.g. specimens). As a zone is only a label, vials can belong to more than one zone. This happens if protocols expect different buffers in a certain position.

In the *InsituPro VS* software you will find four different types of zones. Their tasks are:

- multi use:** allows to draw multiple aliquots of buffers from the same zone (e.g. all buffer vials)
- single use:** vials in the target zone receive a single aliquot only of buffers from corresponding vials in this zone (e.g. probe vials)
- programmed:** the specimen tray is a programmed zone with the number of specimens loaded defined at start. This allows to run the instrument with a specimen tray or slide incubation tub that is only filled partially
- dependent:** some zones of the specimen tray and the slide incubation tub are defined to be dependent. Here the number of specimens or slides entered for the entire (programmed zone) specimen rack or slide incubation tub tells the dependent zone how many specimens or slides it holds.

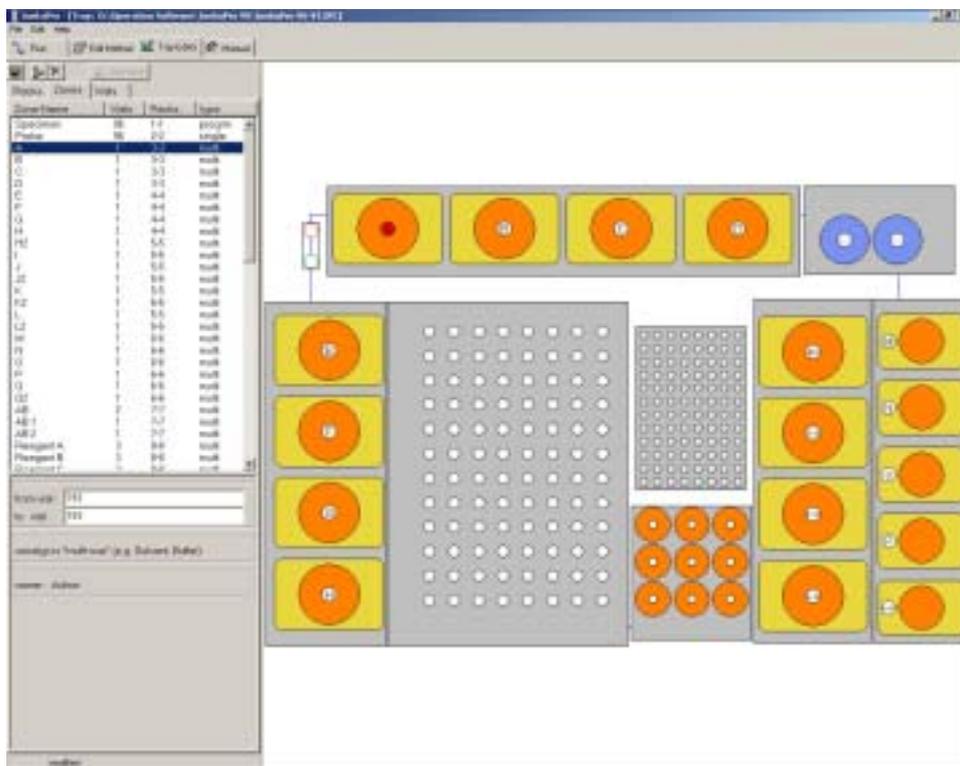
The example displayed on the next page may help to explain this concept.



## Configuration of the work area

For this virtual experiment, the entire specimen rack (programmed zone “Specimen”) is loaded with 12 specimens only. The first row and half of the second row of baskets are filled. Within task 3 the instrument delivers buffer to all baskets. Task 4 fills buffer into the first row of baskets only, defined as a zone called “Basket 1-8”. The fifth task aliquots buffer to the zone “Basket 9-16” (second row of baskets), but this zone is filled only half. As the programmed zone “Specimen” is set to proceed 12 baskets only, it teaches the dependent zone “Basket 9-16” that only its first four baskets are used. As a result, only four baskets of the second row will receive buffer, though the dependent zone covers all baskets in this row.

The type of a zone is displayed in the last column of the zone listing and in the menu bar “Zone Type” when the zone is highlighted.



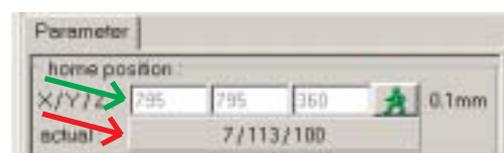
### 7.3 Adjustment of rack coordinates



#### **WARNING**

**Only the system administrator has access to this function. If the instrument does not hit the position of one or more vials properly, please ask your administrator to re-adjust the rack coordinates.**

Several inaccuracies in the rack positioning can add up and require an adjustment from the original ones set during installation. After some time of operation or change of the needle some fine adjustment may be required, too.



To re-adjust the home position of a rack:

- Open the Tray Editor with the instrument connected and switched on
- Open the Racks card
- Select the rack to be adjusted by clicking on its graphics or the definition line in the list
- Verify, that you have selected the right rack
- Move the needle to the desired home position and compare the actual coordinate values (see red arrow in fig. page before) with those displayed as the rack home position (see green arrow in fig. page before). There should be only minor differences.
- If the difference between the preset value and the actual one is bigger than 5 units update the rack home coordinate by typing in the desired values (actual) in the respective field (rack home position, green arrow, fig. page before).

The needle should always aspirate at a safe distance from the vial bottom or the slide. Safe margins are:

- 10 units (= 1 mm) for the teardrop shaped cavities of the specimen tray
- 20 units (= 2 mm) for the slide chambers
- 10 units (= 1 mm) for the 14 ml reaction tubes
- 20 units (= 2 mm) for 50 ml Falcon tubes
- 30 units (= 3 mm) for 125 and 250 ml plastic vials

Always check the changes using the GO button and make sure to save the changes. Move the dual needle downwards to verify that there is some space left.

### Reminder “Rack Home Position”:

The “home position” is defined as the coordinates of the rear left vial in each rack. X coordinates increase from left to right, Y coordinates from rear to front and Z coordinates from top to bottom. The units are 1/10 millimeters. Please refer to Appendix E for the standard home coordinates of all racks.

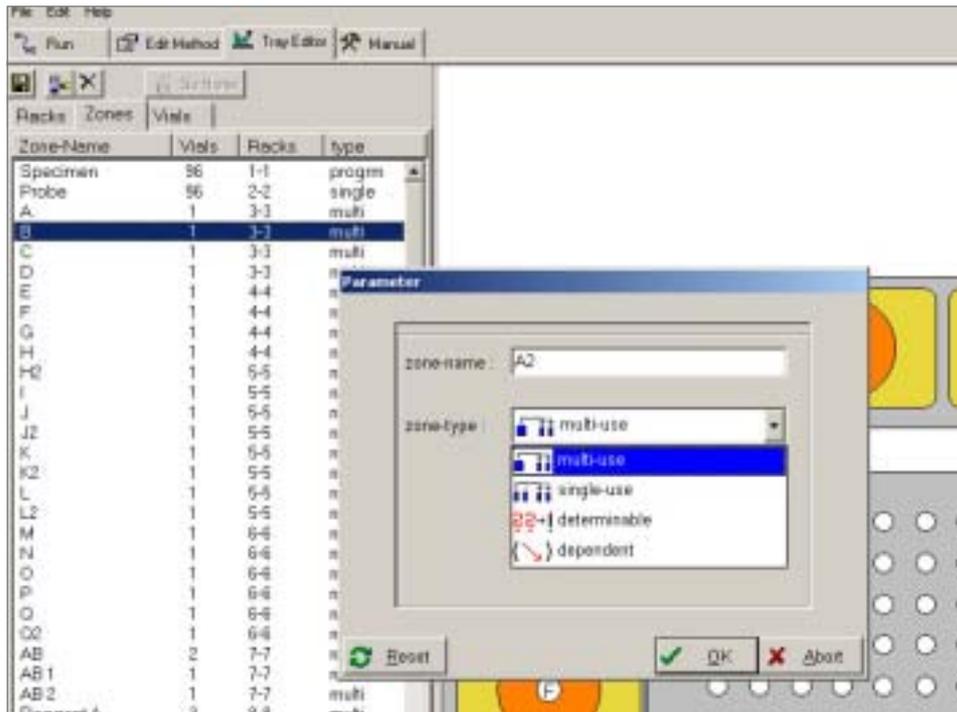
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#### **NOTE**

*The changes are recorded in the Configuration file (ending .IPC). Copies of the program on other computers will not reflect the changes. Therefore, only perform these physical adjustments on the PC operating the system! Keep in mind that changes affect all methods of all users.*

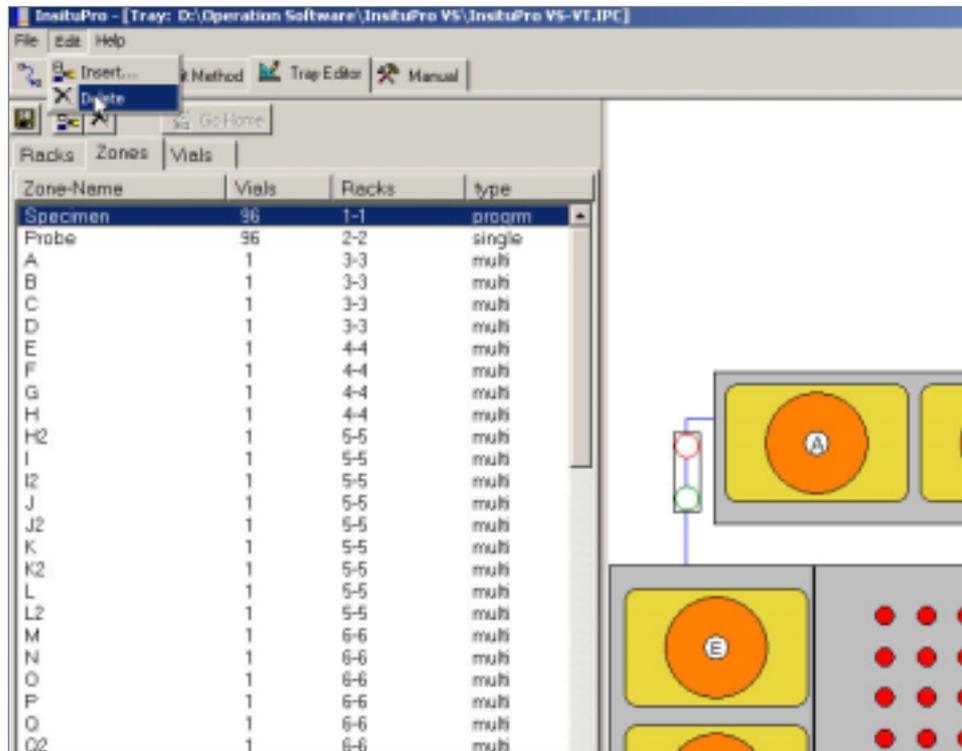
### 7.4 Adding and deleting a zone

The **InsituPro VS** software already includes predefined zones allowing to adapt most manual protocols. Therefore, you rarely have to add or delete zones. However, if e.g. the number of buffers used for your manual method exceeds the number of buffer positions available, you have to define a second zone on top of an existing one. For example to add a Zone A2 proceed as follows:



- Open the file card zone
- Select the zone listed above the one you like to duplicate (Buffer B in this case)
- Now click on "Insert"
- A window will appear (see fig. above)
- Type in the name for the new zone (e.g. A2) and select the type of zone (should always be multi use for buffer vials, see chapter 7.2 for details), then press OK
- The new zone (A2) will appear below the one selected
- Now change the values for "from vial" and "to vial" to the ones for the zone selected before (193 for bottle A). They must be identical!
- Save the changes

A new zone A2 has been created now. It is located at the same position as zone A. You can use this position for two different buffers as both zones will be displayed within the buffer loading form. Do not forget to physically exchange the bottle!



It is not possible to change the name of a zone. To define a zone with your favorite name it may be necessary to delete an old zone first. As all template zones are owned by the administrator, a normal user can only delete the zones created by himself. Proceed as follows:

- Open the file card zone
- Select the zone you like to delete
- Press “Delete” and confirm your decision



**WARNING**

*Deleting a zone used by one of your methods will lead to a failure message (see chapter 6.13). The method can not be started, if it needs a zone that has been deleted.*

7.5 Modifying a zone



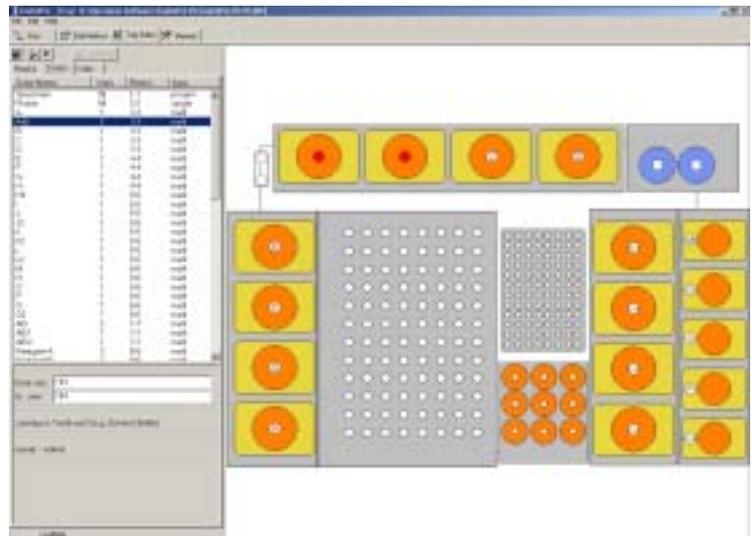
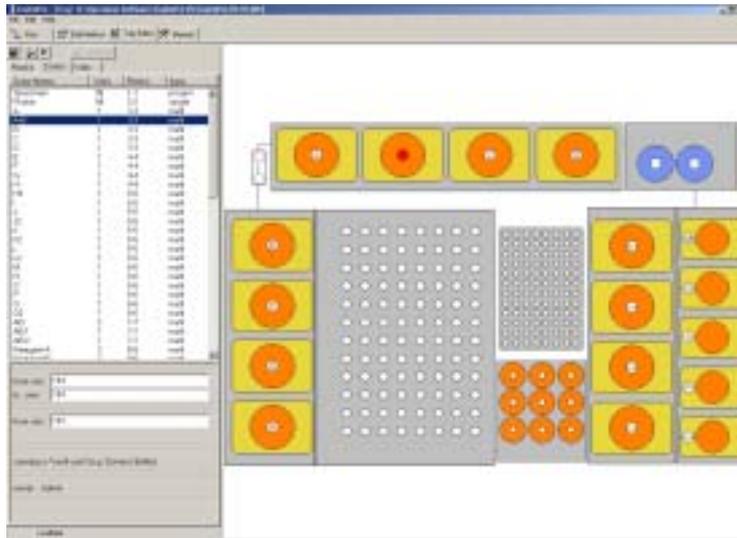
**NOTE**

*Do not modify or delete any of the existing zones as they are used in the pre-defined methods. All method files use the same zone list. To secure this area only the administrator is allowed to modify or delete the basic zones.*

The only feature you can modify for a zone is the number of vials that are grouped in this zone. You may like to change the numbers of vials defined in one of the subzones of the specimen rack or you may like to combine two of the buffer vials (e.g. coupling vials A-B).

## Configuration of the work area

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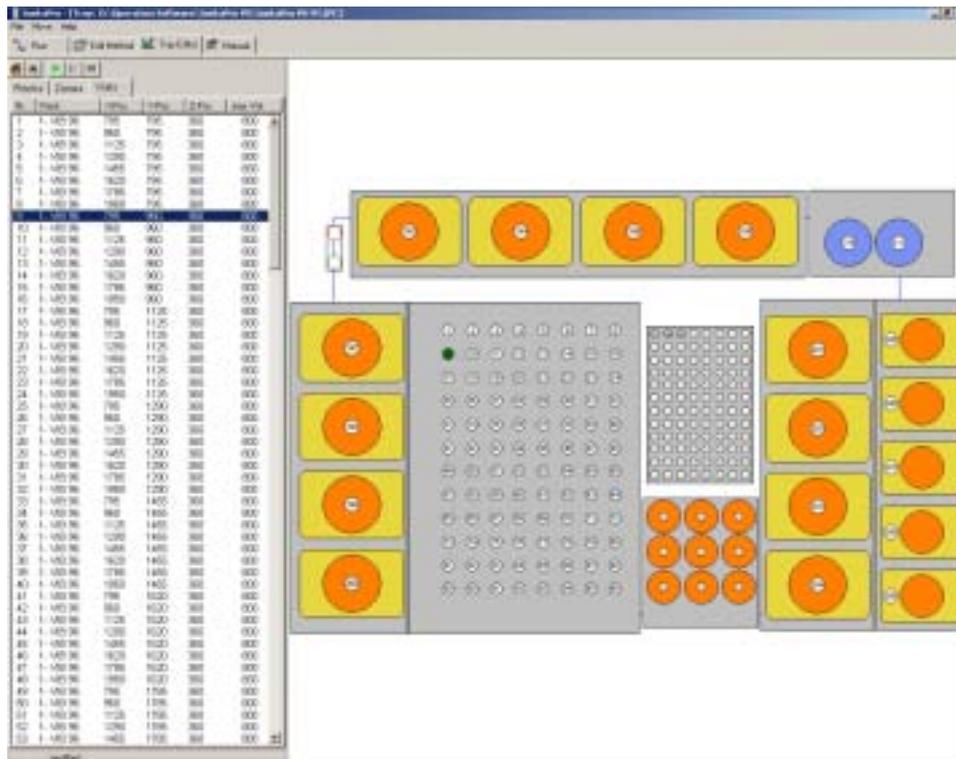
To do this just

- Open the file card Zones
- Create a new zone A-B (you should place it between the position A and B)
- Select the zone A-B you like to modify. Both, the displays “from vial” and “to vial” show 194.
- Now change the value “from vial” to 193 and click in the menu bar “to vial”. The zone A-B will now cover the bottles on position 193 and 194 (shown in the work area display).
- To save these changes go to the “File” menu and select “Save Configuration”. The modified configuration is now saved and valid for all methods using this zone.

The predefined standard zones can not be modified this way, as only the administrator has access to them. If you need to combine buffer vials for your method create a new zone for this purpose. This new zone is valid for your personal method exclusively and can only be modified by you or the administrator.

### 7.6 Check vial position

All vials are consecutively numbered according to the pattern defined for each rack. The operation software determines the physical coordinate for each vial. The list of all vials can be seen by opening the card VIALS. The vial selected in the vial list (right side of the screen) is highlighted on the work area display (left side of the screen).



The main purpose of this menu is to check positions of buffer vials for maintenance purposes. Therefore, you can either move the robot arm to a single vial or to all vials located on the work area. To start a vial check:

- Open the file card “Vials”
- Select the following options either from the menu “Move” or just use the icons displayed above the vial list

**Go home:** Moves the needle to the “home” position, typically over the drain in the rear left corner of the tray

**Go to vial:** Moves the needle to the vial selected. You can select a vial in the list or by clicking in the tray scheme

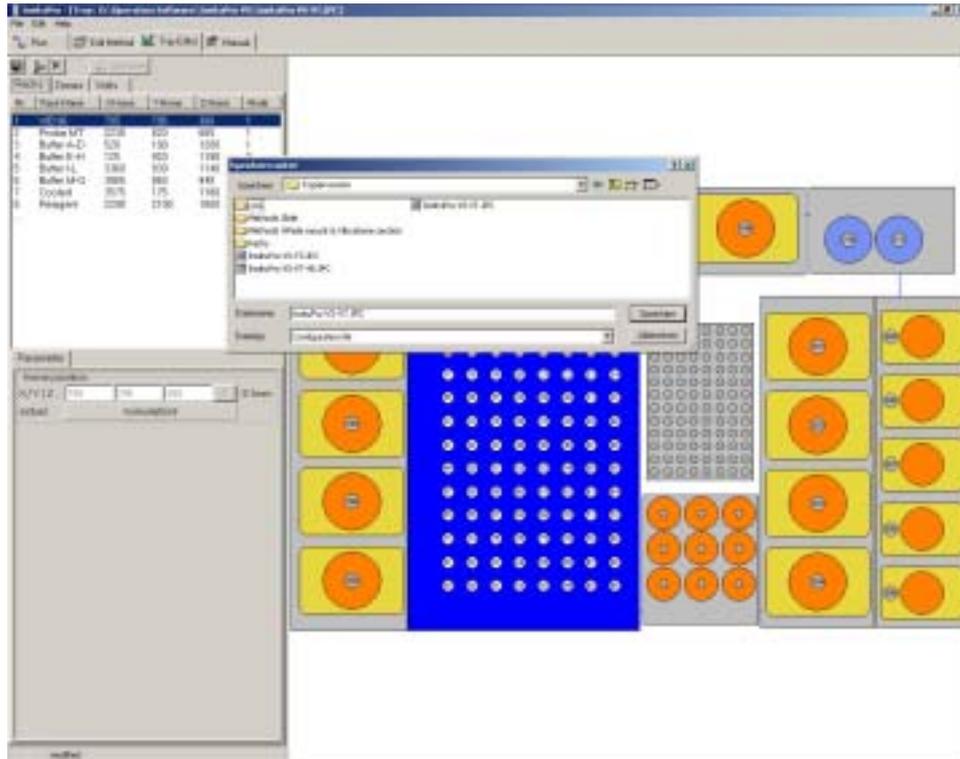
**Check vial position:** Starts an automatic move to all vials, beginning with the selected vial. The routine pauses at the first and last vial of a rack. Continue or stop movement by the control buttons: Next, Pause and Stop

If you move the cursor over a vial on the work area view, a hint-box displays the vial number and the rack. A click on the graphic highlights the corresponding line in the list and vice versa.

## Configuration of the work area

### 7.7 Saving a configuration

As the *InsituPro VS* software handles configuration and method files separately you have to save the configuration after changing parameters within the Tray Editor file card. For this purpose you can either use the “Save” icon or select “Save configuration” in the file menu. If you like to save your private configuration just select “Save as” instead of “Save” and store the configuration with your favorite name.

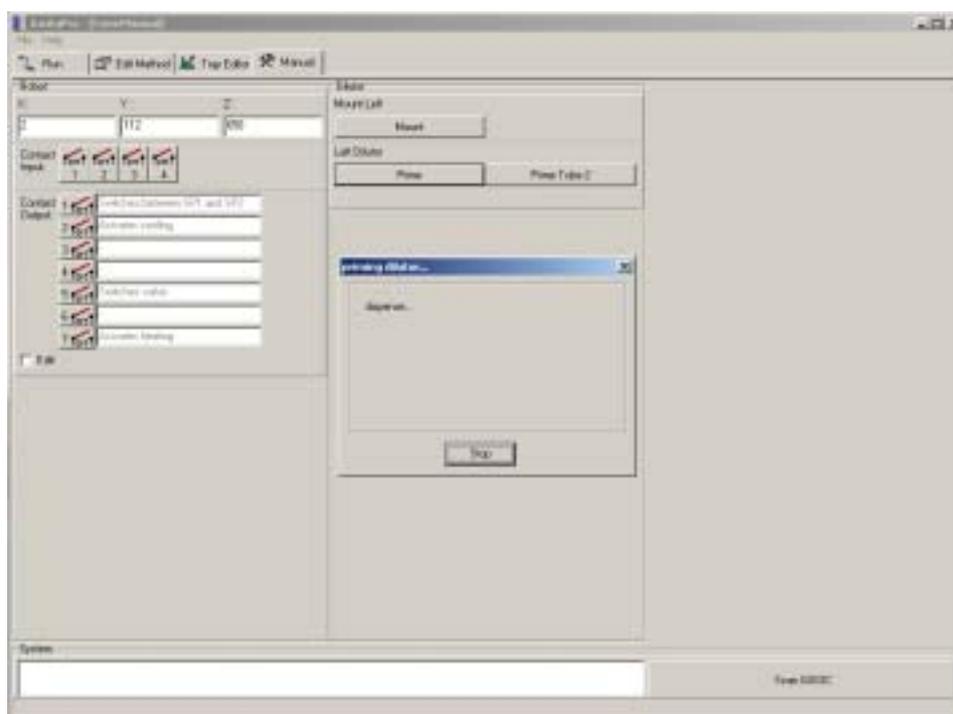


## 8 Manual mode (Manual)

In this chapter the features of the Manual mode are described. It allows to prime both channels for liquid delivery as well as to change the 10 ml syringe, if necessary. Finally, you may use the Manual mode to check some functions of the *InsituPro VS* main board when it comes to malfunction.

### 8.1 Prime the dilutor

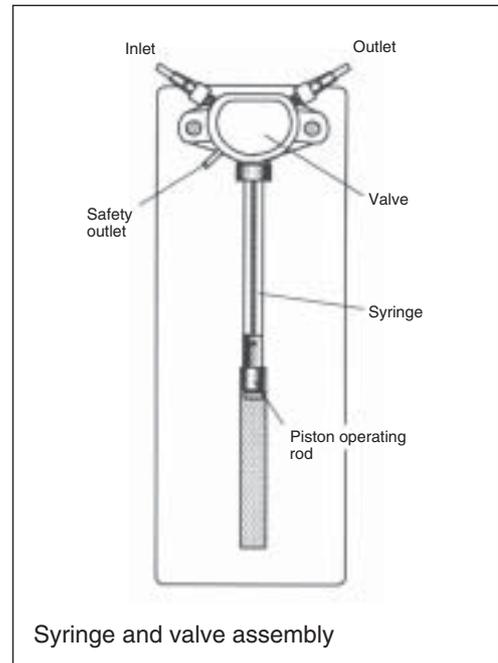
After a long pause (see long term storage of the *InsituPro VS*, chapter 9.2) or if parts of the liquid handling system (syringe, transfer tubes etc.) have been removed, it is necessary to prime both transfer tubes manually. For this purpose you will find a “Prime” function for each transfer tube within the manual mode. To prime a channel just press the appropriate “Prime” button. The syringe piston will start to move to its reset position and the pipetting needle is moved to its home position above the rinse/waste port. The instrument aspirates water from the reservoir bottle into the syringe and dispenses it through the transfer tubes and the dual needle into the waste port. This procedure continues until you click on “Stop”. The instrument continues to prime until the syringe piston has been moved back to its reset position.



### 8.2 Mount a syringe

If the dilutor syringe is damaged or starts to leak, it needs to be exchanged. Switch on the **InsituPro VS** as described in chapter 5.2 and proceed as follows:

- Unscrew the screw fixing the piston to the piston operating rod
- Select the file card Manual of the **InsituPro VS** operation software
- Press “Mount” and wait until the piston operating rod will come to a standstill at its lower position
- Remove syringe and piston completely and insert a new one
- Press OK to move the piston operating rod into its upper position
- Now fix the piston to the piston operation rod and make sure that the screw is tightened.



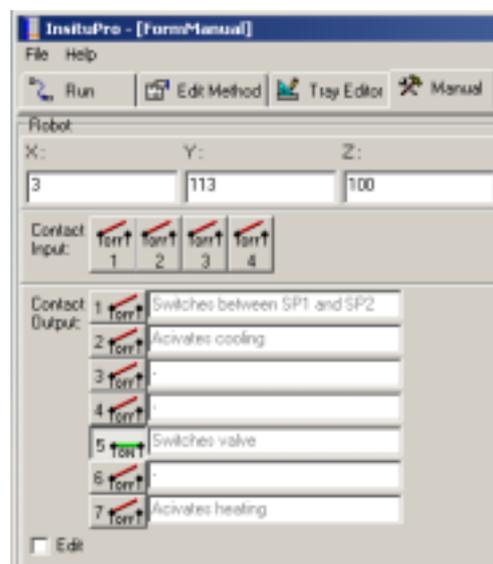
### 8.3 Check the main board

The electronic main board of the **InsituPro VS** is placed in the uppermost part of the instrument not visible for the user. In the upper left compartment where the reservoir bottle is stored, you can see 11 LEDs monitoring the status of the main board.

These LEDs are placed there for diagnosis purpose. If your system shows an electrical malfunction, check these LEDs and call your INTAVIS representative.



To check the main board by watching the LEDs switch on the instrument as described in chapter 5.2. The last five LEDs from the left (No. 7-11) are constantly on. They show that all the different currents needed by the instrument are available. The other six LEDs are used to monitor the functions described in “Appendix A” page 77. To activate them click on the small buttons labeled Contact Output and watch out for the appropriate LED:



Contact	Activates	Monitored by
1	Switches between <b>SetPoint 1</b> and <b>SP2</b> of the thermo controller	Controller shows SP1 / SP 2
2	Activates Peltier cooling of the thermo plate	LED 2 turns green if power is supplied
3	Not used	Clicking noise of the relays
4	Not used	LED 3 turns green
5	Switches 3-way valve for selecting channels of the dual probe	LED 4 turns green
6	Not used	LED 5 turns green
7	Activates heating coils of the thermo plate	LED 1 and 7 turn green

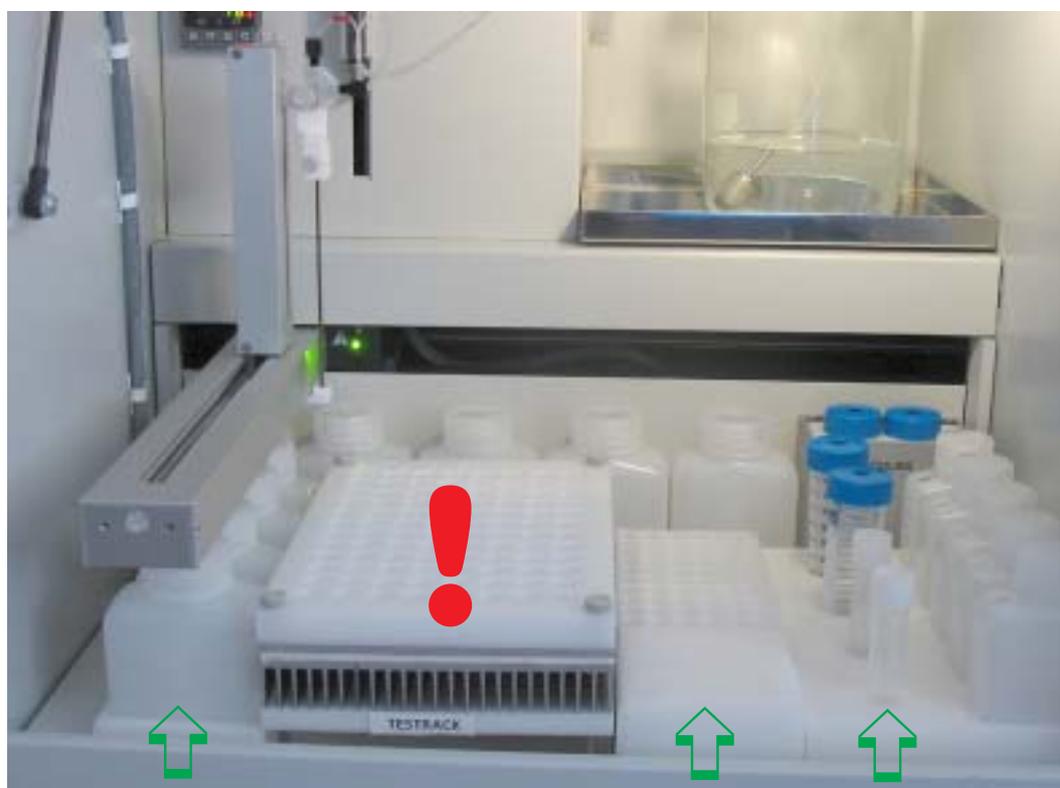


## 9 Maintenance

The *InsituPro VS* needs only little maintenance. However, there are some points you should check routinely to keep the instrument in good shape. This chapter comprises information that will help you to maintain your *InsituPro VS*.

### 9.1 Cleaning the instrument

As continued use of the instrument will leave some traces of salt crystals and other ingredients of the buffers on the work area racks it is necessary to clean these parts regularly. All racks mounted on the large grey plate (see green arrows) can be removed and washed in a dish washer, except the thermo plate (see red proclamation mark), which would not withstand such a treatment.



#### **CAUTION**

##### **Health risk**

*As some buffers used for in situ detection may contain hazardous chemicals, please wear safety glasses, gloves and a protective suit when cleaning the work area.*

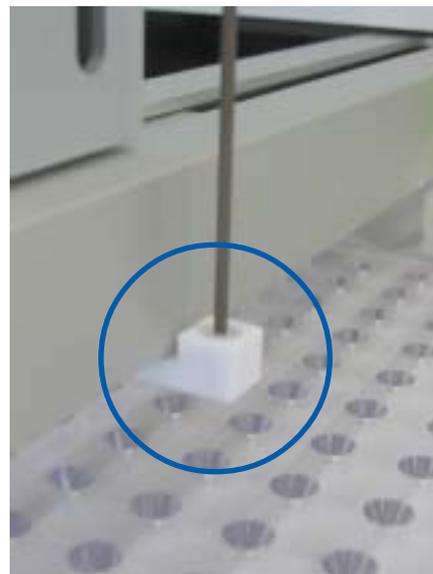
## Maintenance

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The thermo plate, the Peltier cooled buffer rack and all buffer racks mounted behind the grey plate should only be cleaned with a clean cloth dampened with water. If this does not remove all spills you may use a bit of soap to clean these parts.

The needle guide should be cleaned with water from a spray bottle and cloth (blue circle in figure) .

As the “Rinse port” (see chapter 3.2) of the **InsituPro VS** is permanently filled with water, you should clean it at least once a month. Just rinse the port with water from a spray bottle and then rinse it with pure ethanol. This will prevent microbiological contamination of the rinse port.



### 9.2 Preserving the instrument for storage

If you stop to use the **InsituPro VS** for a while (three to four weeks) it is necessary to take some precautions against microbiological contamination of all the parts used for liquid handling. Switch on the instrument as described in chapter 5.2, remove the reservoir bottle and replace it by a bottle containing 500 ml of pure ethanol or isopropanol. Now activate the “Prime” function (see chapter 8.1) and rinse the transfer tube with 30 ml (3 strokes of the dilutor syringe) of alcohol. Stop the dilutor and remove the bottle with alcohol. Reactivate the prime function for both channels and keep it active for at least two to three strokes of the syringe. This step will fill all tubing with air as the inlet filter is not purged in any liquid.

Finally make sure that all buffer bottles used for the **InsituPro VS** are empty, clean the reservoir bottle and store everything in sterile environment. Do not forget to empty and clean the waste bottle at this time. Its content will surely create some strange odors when stored for a while.

### 9.3 Checking screws and fittings

As screws may get loose check from time to time all fittings connecting the liquid handling tubes and the screw fixing the syringe piston rod. The screws to check are:

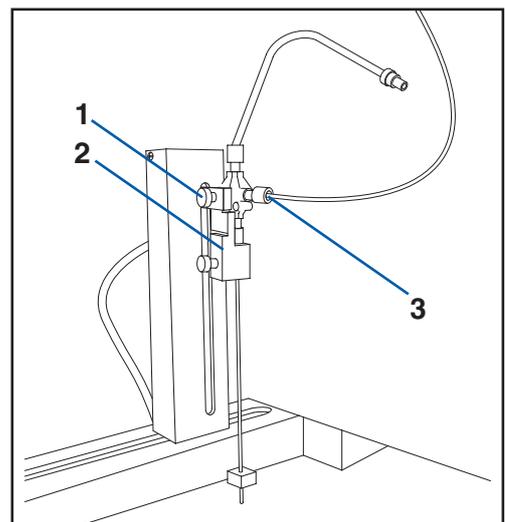
- The screw fixing the inlet filter tube to the dilutor valve (1)
- The screws of the tube connecting the dilutor valve and the 3-way valve (2)
- The screws of both transfer tubes (3)
- The connection between the 10 ml transfer tubes and the pipetting needle extensions (4)
- All screws holding parts of the pipetting needle (5)
- The screw holding the syringe piston (6)



### 9.4 Changing the double needle

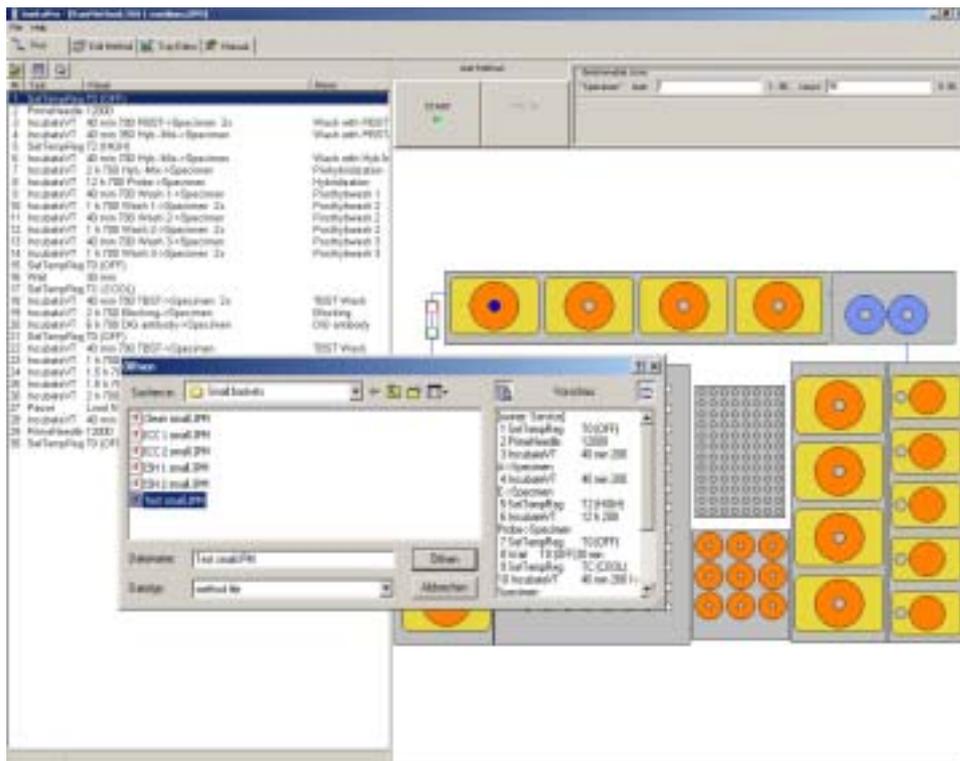
With extensive usage of the **InsituPro VS** some parts may have to be replaced to guarantee a high performance. One of these parts is the pipetting needle used to transfer buffers. To replace it:

- Loosen the screw (1) fixing the needle holder to the Z-arm of the robot
- Remove the needle holder together with the pipetting needle from the Z-arm
- Loosen the screw holding the pipetting needle (2) and pull out the needle
- Unscrew the connection of both transfer tubes to the pipetting needle (3)
- Mount the new needle by just going back the steps explained above.



### 9.5 Carrying out a functional test

A functional test can be carried out, if the system stood still for a long time or if you want to familiarize yourself with the instrument before starting an in situ detection experiment. For this purpose we have created a short test protocol that applies water from several buffer positions to the specimen tray or the slide chambers. The service staff uses this protocol to test the instrument after installation. For details of the protocol please refer to the section “Method development” of the User’s Guide. To run the “Test method”:



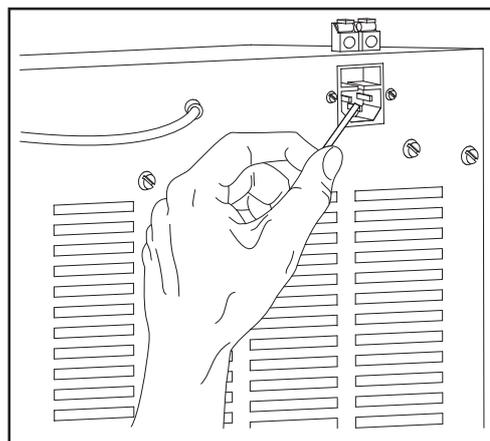
- Switch on the **InsituPro VS** and activate the operation software
- Load the protocol “Test”
- Load sterile water to the positions labeled in the buffer loading form
- Mount the specimen tray or the slide tub with the appropriate lid (no baskets or slides necessary)
- Fill the reservoir bottle with sterile water
- Start the trial and watch the instrument carefully to see if it works properly

### 9.6 Changing the main fuse

A slow blow fuse (2.0 Amp) is installed in the power socket block at the rear of the instrument (see chapter 3.1 for instruments details). If you have to change the fuse be sure to replace it by a fuse of correct type and value. Inside the fuse holder you will find a spare fuse for this purpose.

To change the fuse:

- Disconnect the power cord from the instrument
- Open the fuse holder drawer with a small screw driver and pull it out
- Replace the broken fuse
- Push the fuse holder back into the power socket block
- Connect the power cord and switch on the instrument for a check up.



### 9.7 Cleaning new incubation baskets

As the incubation baskets are not delivered sterile it is necessary to clean them prior to the first use. The most effective way to do this is using the protocol "Clean" (see hard copy within the Method Development section). Just load the baskets into the specimen tray, fill the bottles labeled within the buffer loading form with 3% hydrogen peroxide, water and pure ethanol and run the protocol. The baskets are cleaned up and sterile afterwards, so use them directly or keep them in a clean, sterile place. The sterilization procedure will take about 4 hours.



## 10 Troubleshooting

The *InsituPro VS* is a sturdy instrument assembled from reliable parts. However, as in every system there are several possibilities of unexpected malfunction. This chapter comprises an overview about potential malfunctions, their diagnosis and a potential therapy. The whole section is divided into:

- Electrical problems
- Communication problems
- Mechanical problems
- Application problems

We always try to keep this section as updated as possible. However, especially in case of application problems we recommend to refer to the User Forum offered on our website or to call directly the product specialist.

### 10.1 Electrical problems

**Problem:** Green control light of the power switch stays dark

Possible reason	Solution
Instrument is not switched on	Switch on the system
Power cord is unplugged	Check if power cord is plugged in
No electricity at the power socket	Check the socket for electricity
Fuse is blown	Replace fuse

**Problem:** Green control, LED at the right side of the robot arm does not light up and there is no communication established between the operating PC and the *InsituPro VS*

Possible reason	Solution
Instrument is not switched on	Switch on the <i>InsituPro VS</i>
Main board failure	Check the main board (see chapter 8.3 and Appendix A for details) and contact your local representative
Internal fuse is blown	Contact your local representative

**Problem:** Thermo controller displays “open” at the lower panel or other unexpected values

Possible reason	Solution
Thermo plate or tub is not plugged in correctly	Check the connections “Heating” and “Cooling”
Main board malfunction	Check the main board (see chapter 8.3 and Appendix A for details) and contact your local representative
Thermo controller is defect	Call your local representative

## Troubleshooting

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**Problem:** Thermo plate or tub is not heating or cooling

Possible reason	Solution
Thermo plate or tub is not connected properly	Check the connections “Heating” and “Cooling”
Thermo plate or tub is defect	Call your local representative
Main board malfunction	Check the main board (see chapter 8.3 and Appendix A for details) and contact your local representative

**Problem:** LEDs one and two are dark, all others are on. The system does not establish a communication with the PC.

Possible reason	Solution
Fuse of the XYZ robot is blown	Exchange fuse
Power connection of the XYZ robot is unplugged	Plug in the power cord of the XYZ robot

### 10.2 Communication problems

**Problem:** PC is not able to establish communication with the *InsituPro VS*

Possible reason	Solution
<i>InsituPro VS</i> is not switched on	Turn on the system
Communication cable is not plugged in	Check the communication cable connecting the operating PC and the <i>InsituPro VS</i>
Driver for COM port is not installed properly	Call your local representative
COM port of the PC is not set correctly	Call your local representative
XYZ robot gets no electricity	Check connection and fuse

**Problem:** The system starts to proceed a method but interrupts the run without displaying an error message

Possible reason	Solution
power failure	Check in the log file where the protocol stopped and restart the system at that point
PC went to stand-by mode	Check the energy saver settings of the operating PC. It should not be allowed for the PC to go to stand-by mode
PC is too slow to operate the <i>InsituPro VS</i>	Replace it by a PC with a CPU working at 800 MHz or higher. Operation system should be Windows XP or 2000

### 10.3 Mechanical problems

**Problem:** Dilutor syringe leaks

Possible reason	Solution
Piston does not seal properly	Remove syringe (see chapter 8.3), pull out the piston and clean it up
Sealing of the piston is defect	Replace the syringe

**Problem:** Dual needle is continuously losing buffer or reservoir water

Possible reason	Solution
Syringe is not properly mounted	Check that the syringe is fixed tightly
Tubes are not properly fixed	Check all fittings of the liquid handling system
3-way valve selecting “Waste” or “Fresh buffer channel” is defect	Call your representative for an exchange valve

**Problem:** Uneven volumes are delivered, syringe draws air

Possible reason	Solution
Syringe is not properly fixed in the valve block	Check the syringe and fix it properly
Connections of the liquid transfer tubes are not tight	Check all screws and connections of the liquid handling system (see chapter 9.3 for details)

**Problem:** Uneven volumes delivered, baskets run dry, some buffers are not picked up

Possible reason	Solution
Buffer bottle is not filled or is not put into the work area	Crosscheck buffers loaded with buffer loading sheet
Buffer bottle is too small for the amount of buffer necessary to fulfill the task	Check the buffer consumption with the buffer loading sheet. Insufficient buffer volumes are displayed in red (refer to chapter 5.4 for details)
Screw fixing the dilutor syringe piston is not fixed	Check the screw and fix it
Reservoir bottle is empty, no liquid handling at all	Refill reservoir with water and recalculate the consumption of reservoir liquid with the buffer loading sheet
Racks are not mounted properly. Needle touches the bottom of the vial when aspirating the buffer	Make sure that the buffer racks and the buffer bottles are properly mounted in their appropriate positions
Needle hits vial bottom during aspiration	Check Z drive adjustment (see chapter 7.3 for details)

## Troubleshooting

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**Problem:** Dilutor valve leaks from small stainless steel tube (bottom left)

Possible reason	Solution
Pipetting needle is blocked	Clean pipetting needle
Transfer tube kinked and blocked	Replace transfer tube

**Problem:** Operation stopped and a message like X-drive, Y-drive or Z-drive error appears

Possible reason	Solution
Something hinders the movement of the vertical or horizontal arm of the robot	Check the loading of all buffer vials, check if all bottles used have a pierced cap. Check the alignment of all racks (see chapter 7.3)

**Problem:** Basket cavities are not filled with buffer properly or are overfilled, spillage of buffer over the specimen tray occurs

Possible reason	Solution
Air in the liquid handling system (transfer tubes, syringe etc)	Check the reservoir bottle and prime both transfer tubes manually (see chapter 8.1), check all fittings in the liquid handling lines
Z-drive adjustment is too high or too low	Readjust Z-drive setting (see chapter 7.3)
Buffer volume in source zone is too low	Recalculate the buffer consumption and fill the buffer bottles with the appropriate amount

### 10.4 Application problems

**Problem:** Specimen or tissue is torn into pieces

Possible reason	Solution
Permeation procedure with PK is too stringent	Reduce incubation time or concentration of Proteinase K, try to substitute PK permeation by RIPA buffer or heat treatment

**Problem:** Specimens show no or only weak staining

Possible reason	Solution
Probe is not labeled properly	Check probe labeling
Permeation not sufficient	Test other permeation conditions
pH for staining reaction is not optimal	Check pH of the staining buffer
Concentration of Mg <sup>2+</sup> -ions is too low	Check AP-Buffer recipe
Posthybridization washes is too stringent	Test different wash buffers with reduced stringency
Temperature for hybridization and post-hybridization washes is too high	Reduce temperature for these steps (see chapter 5.7)
Temperature for hybridization is too low, long probe performs self-annealing to form secondary structures	Increase temperature for prehybridization and hybridization
PBST or TBST washes performed at elevated temperature	Allow the thermo plate to cool down after the posthybridization washes for at least 30 minutes before adding PBST or TBST

**Problem:** High background staining

Possible reason	Solution
Prehybridization is too short	Choose a longer prehybridization time (3 hours or more)
Posthybridization washes are not efficient	Increase number of washing steps. If this does not help increase stringency by increasing the temperature or use more stringent buffers for the final washes
Unbound DIG antibody is not washed out sufficiently	Increase the number of washing steps after DIG antibody incubation

**Problem:** Precipitation in prehybridization buffer, probe or posthybridization washing buffers

Possible reason	Solution
SDS precipitation when buffer is stored is too cold	Preheat buffer or preferably substitute SDS by another detergent like e.g. Tween



## 11 List of spare parts

Order No.:

12.100   
pipetting needle, complete

12.104   
12.106   
replacement outer needle,  
replacement inner needle

12.200   
reagent bottle set

12.220   
50 ml reagent vial adapter kit

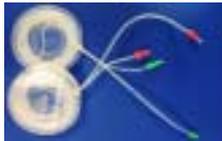
12.230   
12 ml reagent vial adapter kit

12.301   
small basket upgrade kit

12.401   
medium basket upgrade kit

12.501   
large basket upgrade kit

32.060   
10 ml syringe

12.192   
10 ml transfer tubes in a box

12.340   
Small incubation baskets

12.440   
Medium incubation baskets

12.540   
Large incubation baskets

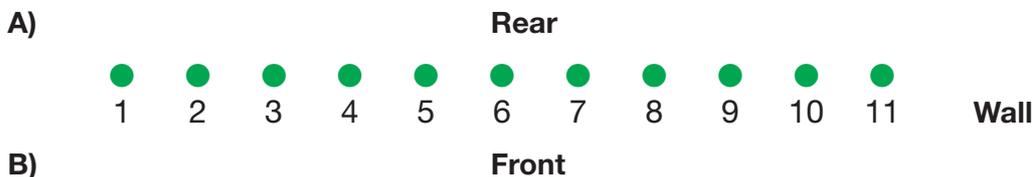
12.906   
Silicon slide holder kit

12.940   
Counter slides



## Appendix A

### LEDs on the *InsituPro VS*



1. Heating on, thermorack supplied with power
2. Peltier cooling of the thermorack active, power supplied
3. Contact Output 4, not used
4. Contact Output 5, switches 3-way valve
5. Contact Output 6, not used
6. Contact Output 7, activates thermo controller output, heating active
7. Internal power supply 5V (DC) active
8. Internal power supply 12 V (DC) active
9. Sampler 12 V power supply (DC) active
10. Transformer 24 V (AC) active, (supplies power for thermorack heating)
11. Transformer 12 V (DC) active, (supplies power for thermorack cooling)

**i**

#### **NOTE**

*To prevent any damage on the transformer the logic board of the InsituPro VS prevents parallel activation of the Peltier cooling and heating of the thermorack.*

*The temperature selection between SP1 and SP2 is not indicated by an LED but only seen on the temperature regulator display. LED 1 and 7 indicate heating for both temperatures.*

*The temperature regulator can switch between two set points T1 and T2. Heating is switched off (room temperature) by setting OPC 7 OFF (LED #6, above). The temperature controller display will still show the last set point, but will not heat!*



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## Appendix B

### Administrator maintenance duties

As mentioned in the introduction the *InsituPro VS* administrator has access to functions of the software that are not open for normal users. Therefore, he/she is the only person who can do an extended maintenance of the instrument.

To keep the *InsituPro VS* in good technical condition, the administrator should check all points listed below within the given time frame. To prevent any damage of the instrument he should make sure that all users are well trained and that the instrument is always handled with care.

#### Monthly duties:

- Check the liquid handling system and make sure that all connections are tight (see chapter 9.3)
- Check the dual probe for accurate Teflon coating
- Make sure that the instrument is kept clean and that consumables are held on stock

#### Quarterly duties:

- Check the vial positions regularly with the check vial position function (see chapter 7.6)
- If necessary, readjust the coordinates for racks as described in chapter 7.3
- Look into the “logfile folder” and erase old logfiles
- Check for updates of the *InsituPro VS* operation software on the INTAVIS website. Availability of a new update will be published in the “News” section of the website. Please contact INTAVIS or your local representative for an update.
- Make sure, that the global settings of the pipetting parameters are according to the users prerequisites (see below).

#### Annual duties:

- Check the file card “Zones” within the “Tray Editor”. If there are too many zones defined that are not used any more erase them
- Check the folders “Methods Whole mounts & vibratome section” and “Methods slides” and erase the files that are not in use any more
- Check the list of users allowed to use the *InsituPro VS* as well as their rights (for details see below)

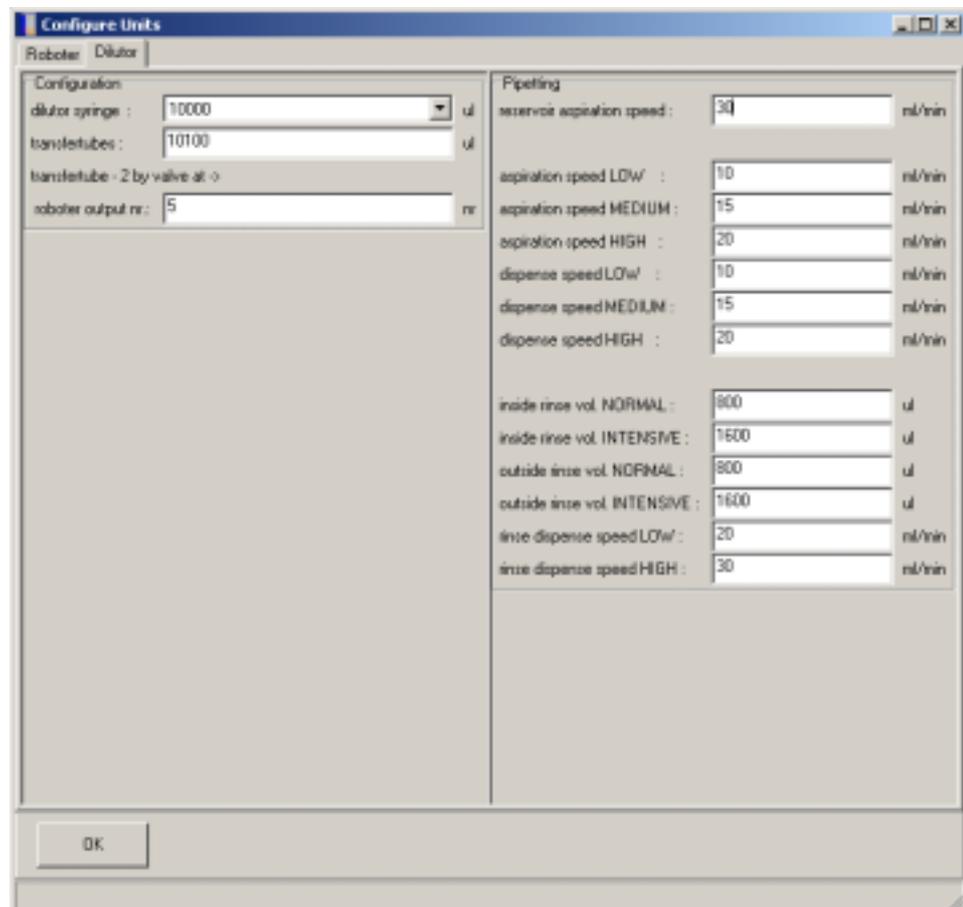
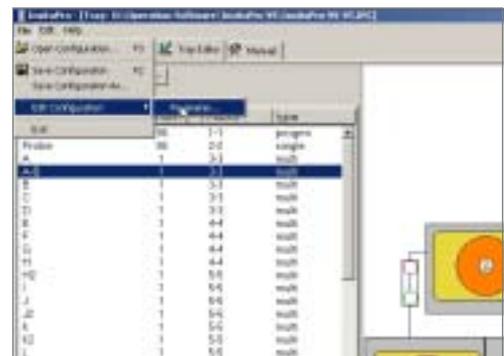
### Additional functions of the software

There are two functions of the *InsituPro VS* software that have not yet been described in the manual, as only the administrator has access to them. One function is the central presetting of pipetting parameters used for liquid handling, the second function is the management of passwords and user lists. Both features will be explained now.

### Definition of global pipetting parameters

The *InsituPro VS* operation software allows to predefine pipetting speed and rinse volumes used for the global liquid handling on a single file card. Changes in these global parameters are rarely necessary. To open this file card:

- Enter the “Tray Editor” and select the menu “File”
- Choose “Edit Configuration” and click on “Parameters”
- The pipetting parameters are displayed now. To optimize them either increase or decrease the values. The table below will help you to decide which pipetting speed will be appropriate for your specific prerequisites



Function	Default	Comments
reservoir aspiration speed	30 ml/min	This is the flow the instrument picks up water from the reservoir. There is no need to change it!
aspiration speed LOW	10 ml/min	If you are working with very viscous solutions you may reduce this value to 5 ml/min. But be aware that this will increase the time the instrument needs to fulfill a complete Incubate task.
aspiration speed MEDIUM	15 ml/min	There is no need to change this value
aspiration speed HIGH	20 ml/min	If you like to speed up the liquid handling you may increase this value. But be aware that some buffers might be too viscous for high pipetting speed
dispense speed LOW	10 ml/min	If you are working with very viscous solutions you may reduce this value to 5 ml/min. Same is true for extremely fragile specimens
dispense speed MEDIUM	15 ml/min	There is no need to change this value
dispense speed HIGH	20 ml/min	If you like to speed up the liquid handling you may increase this value. But be aware that adding buffer at very high speed may harm the specimens.
inside rinse vol. NORMAL	800 µl	Rinsing the needle with one volume of the buffer transferred has been proven to be sufficient. If you like to reduce the consumption of reservoir liquid used, you can reduce this value for the standard buffers. Do not do it for the steps in which probe or primary antibody is added.
inside rinse vol. INTENSIVE	1600 µl	This is normally used to clean the needle intensively after having applied the probe or the primary antibody. It has been shown that using twice of the volume applied to each basket prevents cross contamination. You may increase this value if cross contamination of probes happened.
outside rinse vol. NORMAL	800 µl	See "inside rinse vol. NORMAL".
outside rinse vol. INTENSIVE	1600 µl	See "inside rinse vol. INTENSIVE"
rinse dispense speed LOW	20 ml/min	There is no need to change this value
rinse dispense speed HIGH	20 ml/min	There is no need to change this value

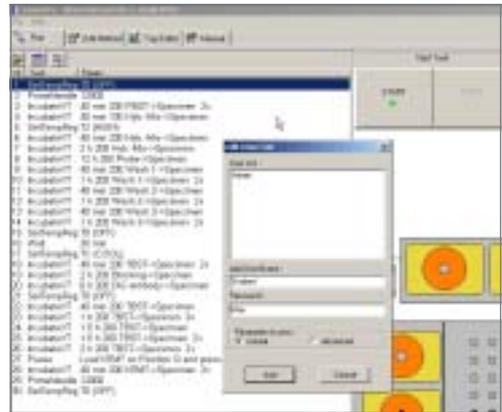
- After optimizing all values press the "OK" button and save the modified configuration as described in chapter 7.7.

### Management of the “User List”

The administrator has the right to delete users, to define new users and to validate their level of access to the software. You can find this function of the software in the “Help” menu. To define a new user:

- Open the menu “File” and select “edit user list”
- Select “add a user” or “delete user” and either:
  - Add a new user e.g. student, select a password and the level of access (normal or advanced)
  - Delete a defined user who does no longer use the instrument
- Press the “Add” or the “Remove” button and confirm changes by clicking on “OK”
- Exit the software now
- When you have defined a new user open the software again and login with the new user’s name and password
- As the software does not know yet which configuration and which method the new user likes to work with, you have to define this now.
- Select the configuration file “InsituPro-VS VT.IPC” (baskets) or “InsituPro-VS TS.IPC” (slides) and open it
- Select an appropriate method from the method folders (“whole mounts & vibratome sections” or “slides”) and load it
- Repeat the same for the other configuration

The level “normal” only allows the user to work with the instrument. He is able to create new methods but has no access to critical parts of the software like the adjustment of racks. A user with “advanced” access to the software has some more rights but still not the level of an administrator. Details have been discussed during the installation of the instrument and are noted down in the service section of the “User’s Guide”.



## Appendix C

### Log file listing of a test method (shortened):

08.07.2004 17:44:52 \* START Method \*

---

— Software : InsituPro — Version: 1.120 / 24.06.2004  
 — GSIOC : Drv:GS\_1.03, DLL:2.01 [32]  
 — Method : Test medium.IPM

---

DATE	TIME	TASK
08.07.2004	17:44:52	<b>1</b> SetTempReg - T0 (OFF)
08.07.2004	17:44:52	<b>2</b> PrimeNeedle - 12000 - Vial: 1 / 1..1
08.07.2004	17:44:52	step 1/2
08.07.2004	17:45:47	step 2/2
08.07.2004	17:46:42	<b>2</b> PrimeNeedle - 12000 - Vial: 1 / 1..1
08.07.2004	17:46:42	step 1/2
08.07.2004	17:47:37	step 2/2
08.07.2004	17:48:32	<b>3</b> IncubateVT - 40 min 700 Water->Specimen - Vial: 1 / 1..96
08.07.2004	17:48:32	dispense into vial 1 to 13
08.07.2004	17:53:01	dispense into vial 14 to 26
08.07.2004	17:57:30	dispense into vial 27 to 39
08.07.2004	18:01:59	dispense into vial 40 to 52
08.07.2004	18:06:28	dispense into vial 53 to 65
08.07.2004	18:10:56	dispense into vial 66 to 78
08.07.2004	18:15:24	dispense into vial 79 to 91
08.07.2004	18:19:54	dispense into vial 92 to 96
08.07.2004	18:21:45	RinseNeedle - NORMAL / NORMAL
08.07.2004	18:22:01	RinseNeedle - NORMAL / NORMAL
08.07.2004	18:22:19	remaining Incub.time: 00 : 06 : 13.4
:	:	:
09.07.2004	09:10:44	<b>12</b> IncubateVT - 40 min 700 Water->Specimen - Vial: 1 / 1..96
09.07.2004	09:10:44	dispense into vial 1 to 13
09.07.2004	09:15:41	dispense into vial 14 to 26
09.07.2004	09:20:41	dispense into vial 27 to 39
09.07.2004	09:25:38	dispense into vial 40 to 52
09.07.2004	09:30:35	dispense into vial 53 to 65
09.07.2004	09:35:34	dispense into vial 66 to 78
09.07.2004	09:39:46	ABswitch to vial 1
09.07.2004	09:40:37	dispense into vial 79 to 91
09.07.2004	09:44:37	ABswitch to vial 1
09.07.2004	09:45:42	dispense into vial 92 to 96
09.07.2004	09:47:17	ABswitch to vial 1
09.07.2004	09:47:32	ABvial 1 is full !
09.07.2004	09:47:56	RinseNeedle - NORMAL / NORMAL
09.07.2004	09:48:12	RinseNeedle - NORMAL / NORMAL
09.07.2004	09:48:30	remaining Incub.time: 00 : 02 : 14.1
09.07.2004	09:50:44	<b>13</b> PrimeNeedle - 12000 - Vial: 1 / 1..1
09.07.2004	09:50:44	step 1/2
09.07.2004	09:51:39	step 2/2
09.07.2004	09:52:33	<b>13</b> PrimeNeedle - 12000 - Vial: 1 / 1..1
09.07.2004	09:52:33	step 1/2
09.07.2004	09:53:28	step 2/2
09.07.2004	09:54:23	<b>14</b> SetTempReg - T0 (OFF)

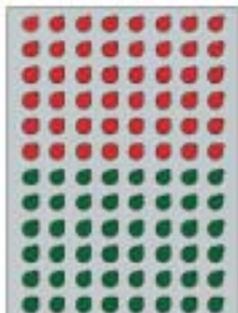
---

09.07.2004 09:54:23 \* END program \*

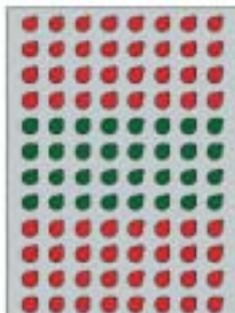


## Appendix D

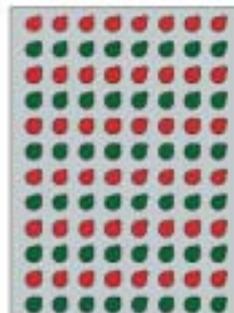
### Predefined “dependent” zones on 96 basket work area:



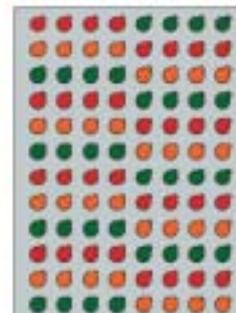
Basket **1-48**  
& **49-96**  
2 zones  
min time: 20 min



Basket **1-32**,  
**33-64** & **65-96**  
3 zones  
min time: 12 min

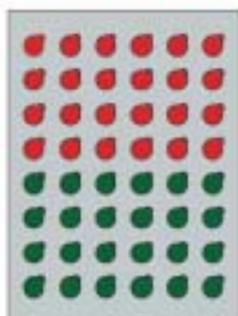


Basket **1-8**,  
**9-16**, etc.  
12 zones  
min time: 4 min

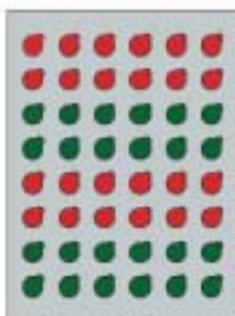


Basket **1-4**,  
**5-8**, **9-12** etc.  
24 zones  
min time: 2 min

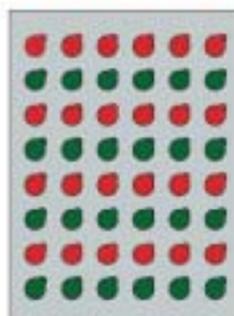
### Predefined “dependent” zones on 48 basket work area:



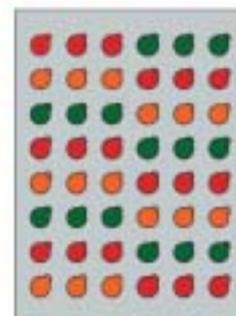
Basket **1-24**  
& **25-48**  
2 zones  
min time: 14 min



Basket **1-12**,  
**13-24** etc.  
4 zones  
min time: 7 min

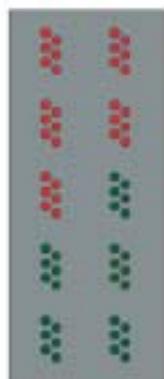


Basket **1-6**,  
**7-12**, etc.  
12 zones  
min time: 4 min

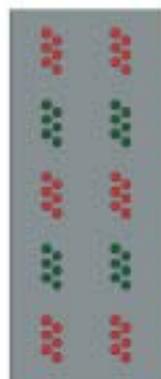


Basket **1-3**,  
**4-6**, **7-9** etc.  
24 zones  
min time: 2 min

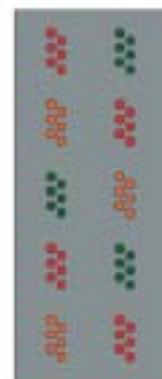
### Predefined “dependent” zones on 60 slide work area:



Slide **1-30**  
& **31-60**  
2 zones  
min time: 3 min



Slide **1-12**,  
**13-24** etc.  
5 zones  
min time: 2 min



Slide **1-6**,  
**7-12**, **13-18** etc.  
10 zones  
min time: 1 min



## Appendix E

### Rack home coordinates

Rack name	Coordinates			Used for
	X	Y	Z	
Vib 48	830	815	380	InsituPro VS large baskets
Vib 96	795	795	380	InsituPro VS 96 small or medium baskets
Slide 10x6	1105	790	300	InsituPro VS slides
Probe MT	2230	810	665	InsituPro VS 96 small or medium baskets
Probe 48	2200	660	1045	InsituPro VS 48 large baskets
Probe 60	2200	660	1045	InsituPro VS slides
Prime Port	1300	1125	1050	InsituPro VS slides
Buffer A-D	510	100	1190	InsituPro VS all configurations
Buffer E-H	105	930	1180	InsituPro VS all configurations
Buffer I-L	3380	930	1160	InsituPro VS all configurations
Buffer M-Q	3860	845	950	InsituPro VS all configurations
Cooled	3545	175	1165	InsituPro VS all configurations
Reagent	2280	2075	1000	InsituPro VS all configurations

The coordinates given in the table above are standard settings of the instrument. They may vary by 2-3 mm (20-30 units) from instrument to instrument.



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