



# Roboinject

## USER MANUAL

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## GENERAL INFORMATION

### Important Safety Advice



Warning: Make sure to read the following advice prior to installation or use of the device and the software. If you do not fulfill all requirements stated below, this may lead to malfunctions or breakage of connected hardware, or even fatal injuries.



Warning: Always obey the rules of local regulations and laws. Only qualified personnel should be allowed to perform laboratory work. Work according to good laboratory practice to obtain best results and to minimize risks.



Warning: The device and the software are not intended for medical uses and must not be used on humans. MCS assumes no responsibility in any case of contravention.

The product has been built to the state of the art and in accordance with recognized safety engineering rules. The device may only

- be used for its intended purpose
- be used when in a perfect condition.

Improper use could lead to serious, even fatal injuries to the user or third parties and damage to the device itself or other material damage.

Malfunctions which could impair safety should be rectified immediately.

### Grounding

This product is grounded through the grounding conductor on the power cord. To avoid electric shock, the grounding conductor must be connected to earth.

### Orient the Equipment Properly

Do not orient the equipment so that it is difficult to manage the disconnection device.

### High Voltage

Electrical cords must be properly laid and installed. The length and quality of the cords must be in accordance with local provisions.

Only qualified technicians may work on the electrical system. It is essential that the accident prevention regulations and those of the employers' liability associations are observed.

Each time before starting up, make sure that the power supply agrees with the specifications of the product. Check the power cord for damage each time the site is changed. Damaged power cords should be replaced immediately and may never be reused. Check the leads for damage.

Damaged leads should be replaced immediately and may never be reused. Do not try to insert anything sharp or metallic into the vents or the case. Liquids may cause short circuits or other damage. Always keep the device and the power cords dry. Do not handle it with wet hands.

## **Guarantee and Liability**

The general conditions of sale and delivery of Multi Channel Systems MCS GmbH always apply. They can be found online at <https://www.multichannelsystems.com/sites/multichannelsystems.com/files/documents/Terms%20and%20Conditions.pdf>

Multi Channel Systems MCS GmbH makes no guarantee as to the accuracy of any and all tests and data generated by the use of the device or the software. It is up to the user to use good laboratory practice to establish the validity of his / her findings.

Guarantee and liability claims in the event of injury or material damage are excluded when they are the result of one of the following:

- Improper use of the device.
- Improper installation, commissioning, operation or maintenance of the device.
- Operating the device when the safety and protective devices are defective and/or inoperable.
- Non-observance of the instructions in the manual with regard to transport, storage, installation, commissioning, operation or maintenance of the device.
- Unauthorized structural alterations to the device.
- Unauthorized modifications to the system settings.
- Inadequate monitoring of device components subject to wear.
- Improperly executed and unauthorized repairs.
- Unauthorized opening of the device or its components.
- Catastrophic events due to the effect of foreign bodies or acts of God.

## **Operator's Obligations**

The operator is obliged to allow only persons to work on the device, who

- are familiar with the safety at work and accident prevention regulations and have been instructed how to use the device;
- are professionally qualified or have specialist knowledge and training and have received instruction in the use of the device;
- have read and understood the chapter on safety and the warning instructions in this manual and confirmed this with their signature.

It must be monitored at regular intervals that the operating personnel are working safely. Personnel still undergoing training may only work on the device under the supervision of an experienced person.

## **Terms of Use for the Roboinject**

You are free to use Roboinject for its intended purpose. You agree that you will not decompile, reverse engineer, or otherwise attempt to discover the source code of the software.

## Requirements for the Installation

Make sure that the device is not exposed to direct sunlight. Do not place anything on top of the device, and do not place it on top of another heat producing device, so that the air can circulate freely.

## Explanation of the Symbols used



Caution / Warning



DC, direct current

## Requirements

- The Roboinject weighs more than 23 kg. Always grip it tightly and do not carry it alone, but with the aid of another person.
- The movement of the well plate carrier can lead to vibrations of the workbench on which the Roboinject is set up. Therefore, the Roboinject must be set up on a rigid, vibration-free base. The base must also be sufficiently solid to carry the weight of the device.
- The Roboinject should be operated only in an air conditioned room. A room temperature of 20° C (or less) is recommended. Make sure that the device is not subject to direct sunlight. It may overheat.
- If the air cannot circulate freely around the external power supply, the device may overheat. Do not shield the power supply by laying anything on top of it.
- The external power supply unit is only for use with the Roboinject. Do not connect it to any other instrument.

## Compressed Air Supply

Even small amounts of water in the compressed air can lead to a corrosion of the carrier. Other contaminations can also result in malfunctions. Use only compressed air fulfilling the following standards.

- The air must be absolutely free of water, oil, and any particles.
- When air is compressed, the humidity in the air is concentrated into a smaller area, frequently condensing inside the air hoses. Use a water separator to remove water from the air. The provided water separator is not suitable to establish the necessary conditions; it serves only as a control device. If you regularly observe an accumulation of water in the provided water separator, the compressed air does not fulfill the required standards.



**Warning: Water in the system leads to corrosion and destroys the device! This is extremely important!**

Use a filter with 5 µm pores or smaller to remove any particles from the air.

- Set the external air pressure at least to 5 bar, the maximum pressure of 10 bar must not be exceeded. Adjust the provided pressure regulator to 3 bar, too high or too low pressure can lead to malfunction and can severely damage the device. Check the pressure at the end of the pressure line, directly before it is lead into the Roboinject. The Roboinject needs a minimum flow rate of 20 l/min.
- Do not use other compressed air hoses than those provided, that is, ID 4 mm OD 6 mm from compressor to water separator, and ID 2.5 OD 4 mm from water separator to the Roboinject. The use of other hoses could lead to a decrease of pressure.

## Handling of the Carrier

- Do not try to lift the carrier off the x/y table. You may move the carrier carefully sideways by hand if necessary but not during operation. For cleaning or alignment purposes use the appropriate software control, please read chapter "Maintenance" and "Alignment".
- If it happens that any liquid spills over the x/y table, please remove it as soon as possible in order to prevent a corrosion of the carrier.

## Handling of the Injector Mount (= z-Axis)

- Do not move the z-axis by hand. Breakage may occur. Always use the software controls to move an axis up and down, please see chapter "Manual Mode".
- Change the injection needle with great care. The injection needle is sharp and may lead to injuries. Stay at a safe distance during operation and protect your eyes. Especially take care not to move your hands in the range of the z-axis.

## Handling of Injection Needles

- The injection needles are sharp and break easily. Always handle them with care.
- In rare cases, a needle may splinter when inserting the plunger into the needle and fitting it. Stay at a safe distance and protect your eyes.
- Dispose the used injection needles into an appropriate container.

## Regular Backups

- You (or the administrator) should perform backups of the Roboinject plate files (\*.rpf files) and template files (\*.rit files) at regular intervals and to appropriate media for preventing data loss. Data loss may be caused by power failure, system and software errors.

## System File Modifications

- If you remove or edit text of an initialization ("ini") or configuration ("cfg") file, the software may cause severe problems. These files relate to hardware functions. A modification of these files may lead to malfunctions or even severe damage of the hardware. Always keep a copy of the original files. Only advanced users should modify program files like these. This warning message applies to all initialization and configuration file modifications.

## WELCOME TO THE ROBOINJECT

### Product Overview



The Roboinject is a fully-automated all-in-one solution for injection of cDNA, mRNA and other compounds into *Xenopus* oocytes in standard 96 well plates. It is also suited for automated injection of other cells or organisms in standard (96, 384) as well as customized well plates.

### Main features:

- Compact and functional design, works fully computer controlled.
- Easy handling - neither special skills nor special equipment required.
- Maintenance-free system.
- Extremely simple and stable mounting of injection capillaries.
- Injection is performed by a positive displacement motorized unit which is capable of repetitively dispensing precisely 1 to 100 nl per cell or organism.
- Reproducible and defined cell impalement depth.
- Automated sample uptake of up to eight different RNAs, DNAs or compounds per well plate from industry standard 0.5 ml reaction tubes.
- Automated and effective needle rinsing before uptake of new samples.
- Injection of up to four different samples per cell.
- Full documentation of all injection parameters.
- Compatibility with the Roboocyte when using 96 well plates.

### Operation Summary

- Instead of having a vast amount of different pull down menus, there is one clearly structured menu bar and also a toolbar presenting commonly used commands. Therefore, you will quickly become familiar with the Roboinject.
- The first step is to set up your "virtual" well plate according to your "real" well plate. Enter a unique ID for the plate and / or assign a file name. The virtual well plate is then visualized in a XY grid (for example, 12 x 8 for a standard 96 well plate) as the real one, and therefore it is easy to overview and to handle.
- Define up to eight different injection samples and select the respective velocity of injection. Enter the respective sample volume.
- Finally, assign the different injection samples to a selection of wells, rows or columns. To allow maximal flexibility, you can inject up to four samples per cell - even on different days - and assign injection depth and injection volume for every cell independently.
- Fill the injection needle with mineral oil and lock it to the injector's luer connector.
- Start the injection sequence by mouse click. The "Injection Wizard" will open and guide you through a quick and easy alignment procedure. Finally, you start the injection and it will proceed automatically until finished or interrupted by the user.
- Information about the plate and the current state of all wells is displayed and updated online on the screen.
- You may save the well plate and load it later on the Roboocyte to proceed with your experiment.

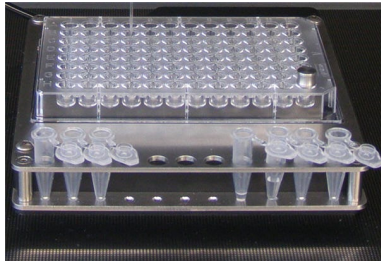


## First Use of the Roboinject

This section contains important information about the general concept of the Roboinject and the setup of the system. Please read the "Important Safety Advice" in the introduction prior to installing the Roboinject hardware and software.

## The Roboinject Hardware

The Roboinject is compatible with standard lab equipment and can be easily integrated in your working environment. Software controls for all settings replace any knobs on the device. The Roboinject is straightforward and easy to operate; handling does not require special skills or special equipment.



The **well plate carrier**, powered by linear motors, hovers smoothly and noise free on a cushion of compressed air above the magnetic x/y table. The carrier operates at a resolution of 20  $\mu\text{m}$ . The complete system does not require maintenance other than occasional cleaning of the steel plate. The well plate carrier can hold up to eight industry standard 0.5 ml reaction tubes serving as sample reservoirs.

The vertically moving **injector mount** (z-axis), holding the injection device, is designed specifically for the demand of high stability, speed and precision. It moves at a resolution of 20  $\mu\text{m}$ ; position and speed are software controlled.



The **injection device** is composed of a linear motor driving a **steel plunger** which fits precisely in the provided injection capillaries. The oil gap between the inner glass wall and the plunger surface serves for a perfect leak tightness and makes additional gaskets dispensable.



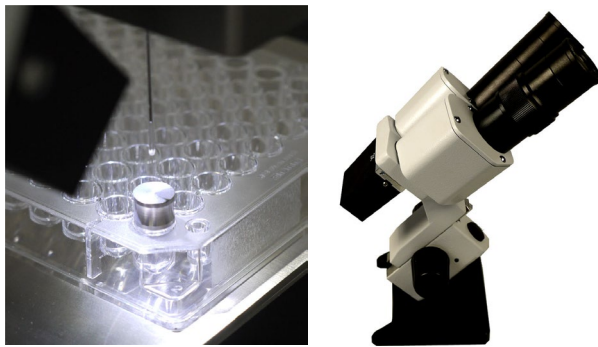
## HARDWARE AND SOFTWARE

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Prepulled borosilicate **glass micropipettes** are provided by Multi Channel Systems MCS GmbH, but custom injection needles may also be used. Mounting of injection capillaries is extremely fast and simple by using luer fittings. There are no additional gaskets susceptible to leakage rendering the injection device durable and maintenance free.

Injection and cultivation of *Xenopus* oocytes is performed using disposable standard 96 well plates, which are commercially available from several providers. Please see chapter "Ordering Information" in the Appendix. The oocytes are plated into the wells in a couple of minutes and can be kept for several days. They quickly settle within the cone-shaped wells and adhere to the well bottom after a few hours. The oocytes do not have to leave the plate anymore; you can easily transfer the oocytes from the incubator to the Roboinject and back again.

A quick alignment process guarantees that the oocytes are injected precisely. The cDNA or mRNA or other compounds to be injected are aspirated into the injection pipette by upward movement of the plunger and then pushed into either the nucleus or the cytoplasm by downward movement of the plunger.



The provided **microscope** is mandatory for the alignment process. Place the microscope in a position onto the x/y table which allows to focus on the **alignment device** plugged in well H12 of the well plate. Please read chapter "Alignment" for detailed information.

## SETTING UP THE HARDWARE

### Setting Up the Roboinject

1. Place the Roboinject on a rigid, stable, and vibration-free base in an air conditioned room.
2. Provide a power supply in the immediate vicinity of the installation site.
3. Provide a compressed air supply in the immediate vicinity of the installation site.
4. Place the computer and its accessories next to it.

### Connecting the Roboinject

The following illustration shows the rear panel of the Roboinject.



Connect all cables as described below.



**Warning:** Carefully lay and secure the cords. Remember that someone could easily trip over a loose cable.

**Note:** All electrical connections are clearly marked, and the plug coding prevents confusion. The cords should be plugged in without the use of excessive force.

### Connecting the Carrier

Plug the carrier connector into the according socket located on the Roboinject's rear panel, labelled with "CARRIER OUT".



**Warning:** Confusion of the compressed air's inlet and outlet may destroy the device. Have a close look at the preceding picture of the Roboinject's rear panel and take care to connect the lines properly. The air hose connecting the Roboinject with the well plate carrier is close to the electrical connection between them.

### Connecting the Roboinject to the Pressure Line

1. Connect one end of the provided compressed air hose (ID 2.5 mm, OD 4 mm) to the water separator.
2. Connect the other end to the compressed air inlet on the Roboinject's rear panel, labelled with "AIR IN".
3. Connect the other provided compressed air hose (ID 4 mm, OD 6 mm) to the provided water separator.
4. Make sure that the pressure line is closed. Connect the free end of the provided compressed air hose (ID 4 mm, OD 6 mm) to the pressure line, labelled with "AIR OUT".
5. Open the pressure line and apply a pressure of 3 bar with the provided pressure regulator.



**Warning:** Make sure that the compressed air used fulfills all requirements for working with the Roboinject. Otherwise, use of an improper compressed air could result in physical harm to the hardware. Especially, check that the compressed air is absolutely free of water, oil, and any particles. Refer to the chapter "Important Safety Advice" for more information.

### Connecting the Roboinject to the Computer

1. Connect the Roboinject by the provided USB cable, labelled with "USB".
2. Connect the Roboinject to the power line.
3. Finally, plug the power cord into the AC power line input, labelled with "24 VDC".
4. Switch the Roboinject on or off via "I/O" toggle switch.



**Warning:** If the air cannot circulate freely around the external power supply, the device may overheat. Do not shield the power supply by laying anything on top of it. Make sure it is not exposed to direct sunlight.

## Installing the Software

### System Requirements

Software: One of the following Microsoft Windows<sup>®</sup> operating systems is required: Windows 10, (32 or 64 Bit), (English and German versions supported).

Hardware: An USB 2.0 High Speed serial bus must be provided for the communication between the Roboinject and the computer. Additional 1 GB RAM working memory is strongly recommended.

Please check the system requirements before you install the software. MCS cannot guarantee that the software works properly if these requirements are not fulfilled.

**Important: Please make sure that you have full control over your computer as an administrator. Otherwise, it is possible that the installed software does not work properly.**

Double-click Setup.exe on the installation volume. The installation assistant will show up and guide you through the installation procedure. Follow the instructions of the installation assistant.

When you have finished the installation of the software and the included driver, you can now operate the Roboinject device.

### Recommended Operating System Settings

The following automatic services of the Windows operating system interfere with the data storage on the hard disk and can lead to severe performance limits. These routines were designed for use on office computers, but are not very useful for a data acquisition computer.

- Turn off "Windows System Restore".
- Turn off automatic "Windows Update".
- Deselect "Windows Indexing Service" for all local disks.
- Turn off "Optimize hard disk when idle" (automatic disk fragmentation).
- It is also not recommended to run any applications in the background when using the Roboinject. Remove all applications from the Autostart folder.

Please check the system requirements before you install the software. MCS cannot guarantee that the software works properly if these requirements are not fulfilled.

**Important: Please make sure that you have full control over your computer as an administrator. Otherwise, it is possible that the installed software does not work properly.**

### Installing the Roboinject Software with Computer connected

**Power up the connected computer and wait until it is ready.**

1. Double-click Setup.exe on the installation volume. The installation assistant will open and guide you through the installation procedure.
2. Follow the instructions of the installation assistant. The Roboinject software and all necessary drivers will be installed on your computer.

## ROBOINJECT SOFTWARE

### Starting the Roboinject

Press the main switch on the back of the Roboinject for switching on the Roboinject. The Roboinject is starting now. After a few seconds the Roboinject is ready for use.

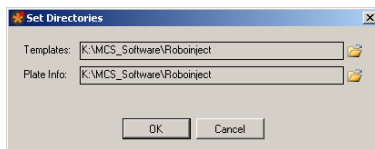
### Starting the Roboinject Software

You may use the computer and the software offline that is, the Roboinject is switched off or not connected to the computer, to review already injected plates or define new injection protocols. You can switch from offline to online mode, when the Roboinject is switched on later. However, if you start the software to operate the Roboinject, the easiest way is to start the Roboinject first and then start the software.

Each time you start the Roboinject software the well plate carrier and the injection mount will automatically move to a reference point, which means to the "home" position. These movements do not use any software command.

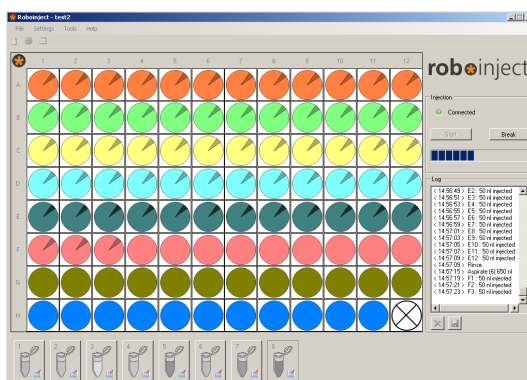
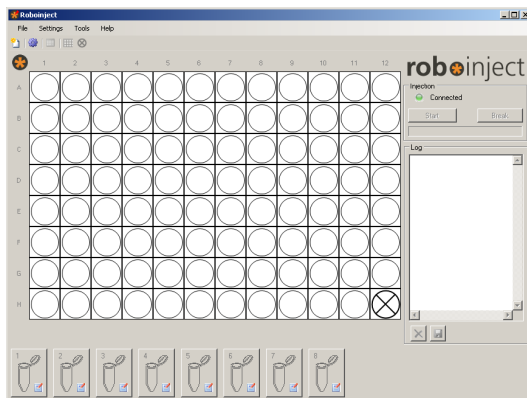
The factory preset default values have been extensively tested with RNA and DNA injection of *Xenopus* oocytes and should work in most circumstances. Therefore, it is advisable to use the default values for the first injections with *Xenopus* oocytes.

1. Double-click the Roboinject icon on the desktop. - OR - Select Roboinject from the "Start" menu to start the Roboinject software.
2. When you start the Roboinject software for the very first time, the file dialog box appears for setting the directories for the plate files and template files. You can either accept the predefined directories or select directories according to your personal preferences.



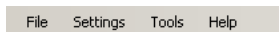
If the Roboinject is offline indicated by a red status lamp below the Roboinject logo, switch the Roboinject on and wait until the status lamp turns green.

The Roboinject Main window opens.



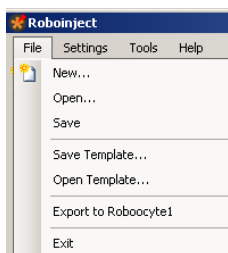
## Main Menu

For maximum flexibility all relevant parameters for the injection can be changed by the user via main menu.



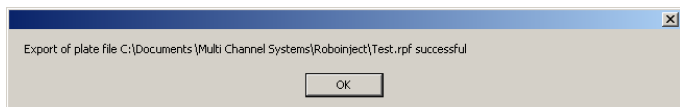
**Note:** The preset default values have been extensively tested with RNA and DNA injection of *Xenopus* oocytes and should work in most circumstances. Therefore, it is advisable to use the default values for the first injections with *Xenopus* oocytes.

## File



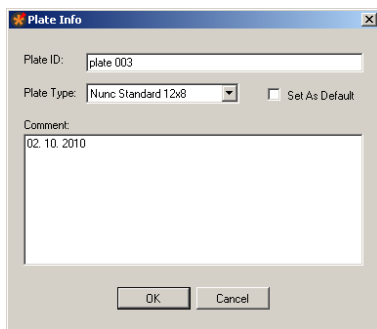
Menu for creating new files, opening existing files, and for saving them. Menu for opening and saving templates, and for closing the program.

The option "**Export to Roboocyte1**" is a feature for exporting plates to the Roboocyte1 device from Multi Channel Systems MCS GmbH.



Well plate files, created and saved with Roboinject software, can be transferred to the data base of Roboocyte1 software. The corresponding well plates, injected with the Roboinject device can be processed with the Roboocyte1 device. The feature is available only for owners of Roboocyte1 devices (Clamp Amplifier A and Clamp Amplifier B) and together with Roboinject software version 1.1.0 which includes a firmware update. It is not possible to export plates to other devices.

Click "**New**" in "File" menu. The following "**Plate Info**" dialog opens.



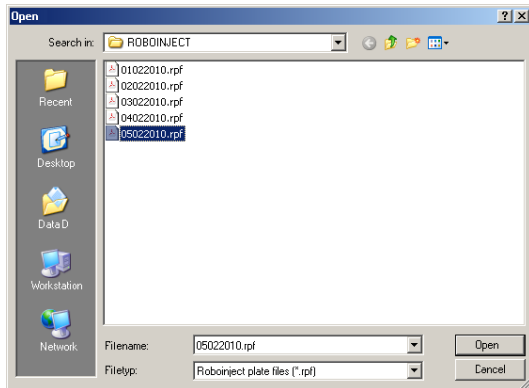
Please give an appropriate file name in "**Plate ID**". The type of the well plate can be defined from the "**Plate Type**" drop down menu. If you want to set the selected plate type as default, please enable the check box "**Set As Default**". You can type in notes concerning the actual experiment into the "**Comment**" text box. All information will be stored in the plate info (\*.rpf) file, and displayed in the report sheet.

If you want that the plate ID is automatically counted up when creating a new file, please enable the check box "Auto increment plate ID" in "General" tab of the "Options" dialog in main menu "Settings".

Click "**OK**" to enter additional dialogs, or to start the injection.

If you want to inject the same plate again or review previously injected cells, open the appropriate well plate file.

Click **"Open"** in "File" menu. The **"Open"** dialog box appears.



Browse your folders and select the Roboinject plate info (\*.rpf) file you want to open. Click **"Open"**. The main window now shows the state of the already saved plate.

Click **"Save"** to save a Roboinject plate info (\*.rpf) file. It will be stored in the selected "Plate Info" directory.

Click **"Save Template"** and **"Open Template"** to open and save a template (\*.rit) file. It will be stored in the selected "Template" directory, respectively.

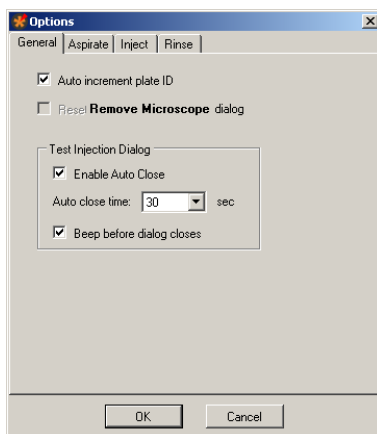
Click **"Exit"** to close the Roboinject software program.

## Settings



Menu for setting options and for defining directories.

Click "Options" in main menu "Settings". The "Options" dialog has four tabs. Click "General" tab.



The dialog allows general settings concerning two general software features, and the "Test Injection Dialog" in particular.

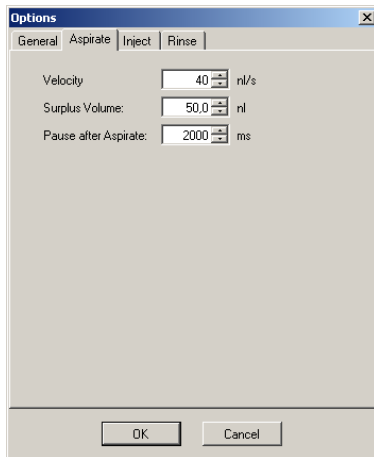
Enable the check box "Auto increment plate ID", if you want that the plate ID is automatically counted up when creating a new file.

Enable the check box "Reset / Remove Microscope dialog" if you want to see the "Remove Microscope" dialog after alignment again.

The "Test Injection" dialog appears immediately before the injection run of each sample if selected in the "Sample Information" dialog. This allows the user to execute a test injection before starting the injection run or to stop the injection run completely.

With regard to the "Test Injection Dialog" you can select or deselect the two check boxes "Enable Auto Close" and "Beep before dialog closes". Additionally, you can choose the time span for displaying the "Test Injection Dialog" from the drop down menu. The "Auto close time" varies from 30 to 180 seconds.

Click "Aspirate" tab.



This dialog allows to define the aspiration of compound from the reservoir tubes.

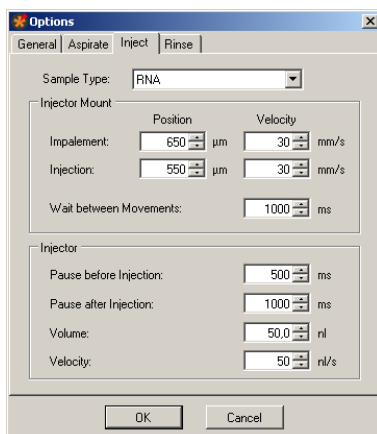
The parameters are the "Velocity" of aspiration in nl/s, the "Surplus Volume" in nl, and the "Pause after Aspirate" in ms.

**"Velocity"**: The aspiration velocity for a given compound depends on its viscosity. Samples with high viscosity should be aspirated slower than samples with low viscosity. This prevents the accumulation of negative pressure inside of the injection needle leading to the formation of air bubbles inside of the capillary.

**"Surplus Volume"**: In order to prevent the injection of minute volumes of mineral oil - the needle was filled with first - with the last injection, you can define a "Surplus Volume". The volume will be aspirated additionally to the injection volume, defined in the "Injection" tab. Values of 50 nl are recommended.

**"Pause after Aspirate"**: You can define a time period during which the injection needle stays in the compound after aspirating the RNA, DNA or other compounds, before the injector mount moves upward. This feature minimizes the risk of sucking air into the needle tip. A time span of 2000 ms is recommended.

Click "Inject" tab.





The dialog allows to define parameters concerning the "Injector Mount" (z-axis) and the "Injector" device itself. The settings are attributed to the different "Sample Types" such as RNA, DNA or others.

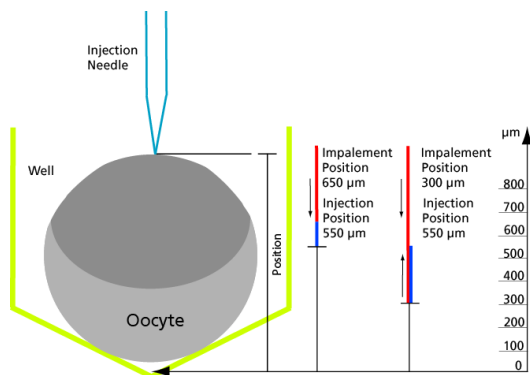
**"Sample Type"**: Define the type of compound to which you want to assign the setting parameters from the drop down menu.

**"Injector Mount = z- Axis"**:

You can separate the impalement process of the injection needle into an oocyte or another cell in two phases. This is especially useful, for example, when working with "soft" oocytes. If the first movement (for example, to 650  $\mu\text{m}$  above well bottom) only pushes down the cell without penetrating the vitelline layer and membrane, a second movement (for example, to 550  $\mu\text{m}$  above well bottom) will most likely lead to a successful impalement. Please see the picture.

Please define the parameters of the first step, called **"Impalement Position"**. Please note that the value in micrometer refers to the distance between the tip of the needle and the well bottom. Define the **"Velocity"** of impalement in mm/s. The default value of 30 mm/s was intensively tested with oocytes and will work in most of the cases.

Please define the parameters of the second step, called **"Injection Position"**. Define the position of the injection in  $\mu\text{m}$  from the up-down control. Please note that the value in micrometer refers to the distance between the tip of the needle and the well bottom in relation to the bottom of the well. Define the **"Velocity"** of injection in mm/s. Again, the default of 30 mm/s will work with most oocytes.



**Example** on the picture: The bottom of the well is value zero, the reference value. If you define, for example an impalement position of 650  $\mu\text{m}$ , the injector mount will move down until its position is 650  $\mu\text{m}$  above the bottom of the well. If you additionally define an injection position of 550  $\mu\text{m}$ , the injector mount will move down again until its position is 550  $\mu\text{m}$  above the bottom of the well.

If you define, for example an impalement position of 300  $\mu\text{m}$ , the injector mount will move down until its position is 300  $\mu\text{m}$  above the bottom of the well. If you additionally define an injection position of 550  $\mu\text{m}$ , the injector mount will move up until its position is 550  $\mu\text{m}$  above the bottom of the well.

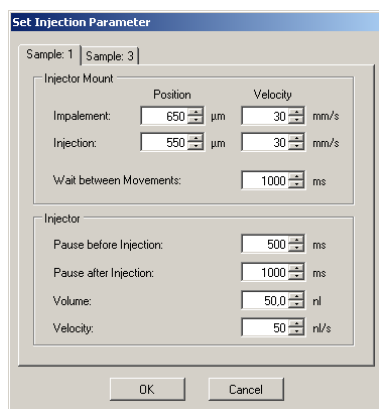
The **"Wait between Movements"** in ms as well as the "Pauses before and after Injection" can be adjusted to user preferences.

## “Injector”

The injection interval is terminated by the time frame before injection "**Pause before Injection**", and the time frame after injection "**Pause after Injection**", both measured in ms. The default values for the wait times before and after injection (500 ms and 2000 ms, respectively) have been tested with oocytes and RNA injections.

You have to define the injection "**Volume**" in nl, and the "**Velocity**" in nl/s from the up-down controls. The respective default values (50 nl and 70 nl/s) have been tested with oocytes and should work in most cases.

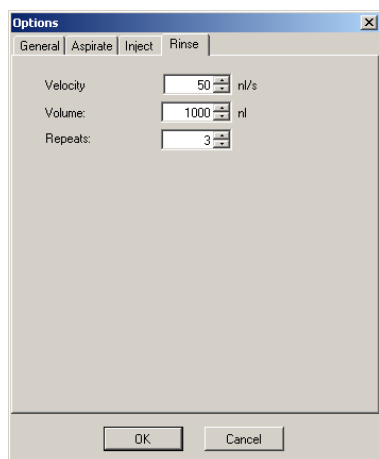
## Adjusting the Injection Parameters for each Well separate



It is possible to adjust the settings for the injection for each single well separate. Click with the right mouse button onto a well in the virtual well plate, and the following dialog appears. Now you can adjust the injection parameters for this specific well and for each sample separate.

If the oocyte in the specific well should be injected with different samples, you can adjust the injection parameters for each sample separately. Please click the "Sample" tabs.

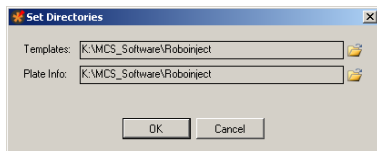
Click "**Rinse**" tab.



Rinsing the injection needle between the injection of different samples is mandatory for preventing cross-contamination. In principle, you can reuse an injection needle as long as it is not clogged or broken. Nevertheless, MCS does not recommend to reuse the needle longer than one day after the last injection.

Define the number of "**Repeats**", the "**Rinse Volume**" in nl, and the "**Velocity**" in nl/s from the up-down controls to avoid contamination when working with different compounds. Volumes of 1000 nl and three repeats guarantee the absolute purity for each injected sample.

Click **"Directories"** in main menu "Settings". The **"Set Directories"** dialog opens.

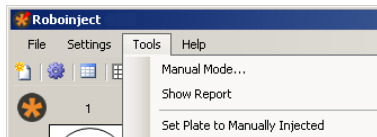


**Templates:** Please browse through your folders, and define the directory you want to store your template files with the extension **"\*.nit"**.

**Plate Info:** Please browse through your folders, and define the directory you want to store your plate info files with the extension **"\*.rpf"**.

The **"Log"** file with the extension **"\*.log"** is a file you can save by users command from the "Log File" window. Please browse through your folders, and define the directory you want to store the log files.

## Tools



Menu for manual mode and for creating a report sheet. The option "Set Plate to Manually Injected" offers the possibility to document information about a well plate, loaded with manually injected oocytes which will be processed with the Roboocyte1 device. Please see below.

About software controlled manual manipulation of the Roboinject, please read the next chapter **"Toolbar" "Open Manual Mode Dialog"**.

Click **"Show Report"**.

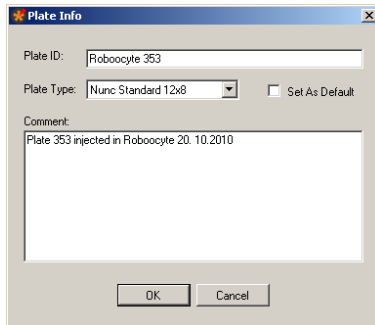
Plate ID:		10022010_1		roboinject				
Plate File:	C:\Program Files\Roboinject\Plate Info\10022010_1.rpf			Report Date:	12.02.2010 09:59:47			
Plate Date:	09.02.2010 14.02.21	Plate Type:	Greiner Standard 12x8					
Comment:								
Samples								
ID	Name	Type	Conc.	Vol. [nl]	Inj. Speed	Comment		
1		RNA		0,0	Standard			
2		RNA		0,0	Standard			
3	test	RNA		0,0	Standard			
Plate								
Well	Sample ID	Sample Name	Inject.	Vol. [nl]	Pen. Depth [µm]	Inj. Depth [µm]	Injection Date	Conc.
A1	1		✓	50,0	600	600	10.02.2010 16:16	
A2	2		✓	50,0	600	600	10.02.2010 16:17	
A3	3	test	✓	50,0	600	600	10.02.2010 16:19	
A4	1		✓	50,0	600	600	10.02.2010 16:16	

The report sheet displays all important information about the selected well plate: Plate ID, plate file destination, report date, plate date, plate type and notes written in the comment. When injection run has finished you find additionally the definition of the samples, and the well plate. The log file is displayed.

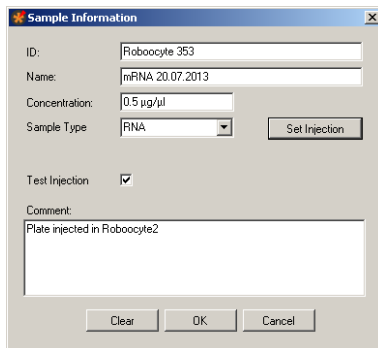
Click **"Set Plate to Manually Injected"**.

When you are using the Roboocyte1 device from Multi Channel System MCS GmbH and you decide to inject manually, or if you performed manual injection with Roboinject for other reasons, you can define the oocytes of a well plate as "injected" with this function.

Click "File" menu "New" to create a new plate. Fill in the respective information about the well plate, created and injected in Roboocyte1.



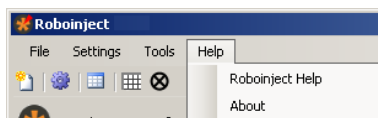
Define the samples which were used for injection in Roboocyte1 device.



Define the respective wells in the plate view.

Right-click on each well to document the information about the injection of the cells in the "Set Injection Parameter" dialog. After that click the option "Set Plate to Manually Injected", and the wells - already injected in Roboocyte1 - are marked as injected with black arrows. After doing so, please save the file. Then you can create and print a report about a well plate, which cannot be created in Roboocyte1 software. The feature is available only for owners of Roboocyte1 devices (Clamp Amplifier A and Clamp Amplifier B) together with Roboinject software version 1.1.0 which includes a firmware update.

## Help



The menu offers the use of the Roboinject online help, and shows the "About Roboinject" dialog.

Click "Roboinject Help" to start the online help.

Click "About".



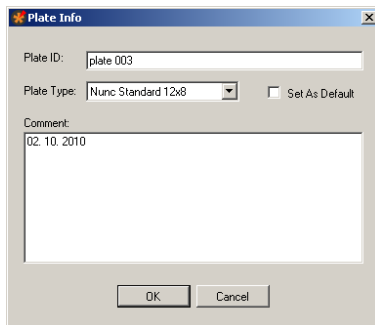
The dialog displays the actual used version of Roboinject software, and hardware information.

**Note: Please keep in mind that these information are essential in case of support!**


## Toolbar

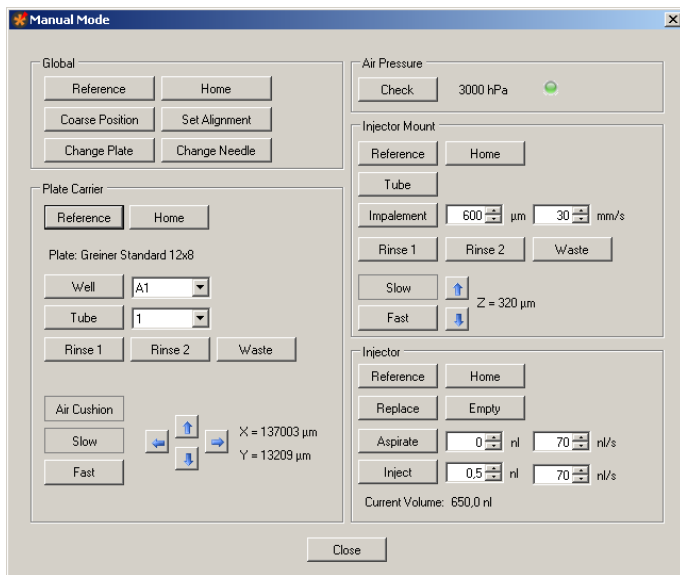


Click the "New File" icon .



The "Plate Info" dialog opens. Please read the description in chapter "File" of the main menu.

Click the "Open Manual Mode Dialog" icon .



The "Manual Mode" dialog opens. The dialog is divided in five sections: Global, Plate Carrier, Air Pressure, Injector Mount and Injector. That means, you are able to influence each part of the device manually via software controls. The commands inside the sections refer to the plate carrier, the injector mount (= z-axis) and the injector (= plunger) only. The "General" section refers to the complete device. The "Air Pressure" check is for measuring the air pressure in the pressure line of the Roboinject device.



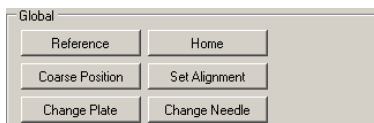
**Warning:** The "Manual Mode" gives you the chance to test the Roboinject device and all its parts separately. Since all kind of movements are allowed, you can easily destroy almost any part of the Roboinject by executing movements at the wrong time. Please always double-check before executing any command, whether this command could be harmful to the Roboinject.

### Examples:

- Moving the carrier when the injection needle is inside of an well will destroy the needle.
- Moving the z-axis to "Tube" position when the carrier is at any well position will destroy the needle and bend the plunger.
- Moving the carrier when the z-axis is at "Tube" position will destroy the injector device and bend the plunger.

Please handle with great care!

### Global Section



Click "Reference" to perform reference movements of carrier, injector mount (z-axis), and injection plunger.

Click "Coarse Position" to initialize the manual carrier and z-axis alignment after installation of the injection needle. The z-axis will move to a position 3 mm above the last z-alignment position, and the carrier will move to the last x/y-alignment position. (If the last alignment was right, this will be exactly below the position of the injection needle).

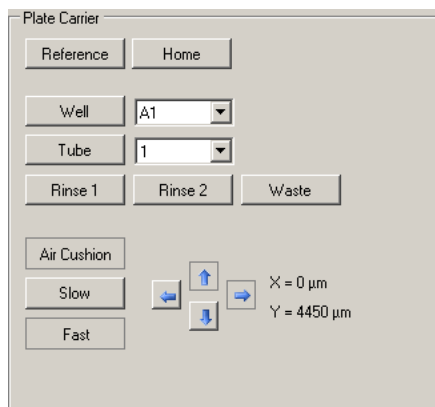
Click "Change Plate" for changing the well plate. The carrier will move to a position at which you can easily access the well plate or the reservoir reaction tubes.

Click "Home" for moving the carrier and the z-axis in home position ( $X = 0$ ,  $Y = 0$ ,  $Z = 0$ ).

Click "Set Alignment" for setting the actual position of carrier and z-axis at the alignment position. Before doing so, please insert the alignment tool in well H12 when using a 96 well plate.

Click "Change Needle" for installing, changing or removing the injection needle in the injection mount. The carrier will move to its home position (or stay there), and the z-axis will move down to allow you an easy access to the luer connector for the injection needle.

## Plate Carrier Section



Click "Reference". The carrier performs its reference movement in x/y direction. If the carrier does not move, please check all cable connections.



**Warning: Perform the alignment procedure before using any of the following commands. Please double-check whether the z-axis is in "Home" position whenever carrier movements are elicited. Otherwise, there is the risk of severe damage to the injector.**

Click "**Home**" for moving the carrier in home position. The home position is defined as the backmost right corner of the x/y table when looking on the front panel of the Roboinject ( $X = 0$ ,  $Y = 0$ ).

Select a "**Well**" from the drop down menu. Select well A1, for example, and click "Well". The carrier will move until well A1 is exactly underneath the injector axis.

Select a "**Tube**" from the drop down menu. Select tube 1, for example, and click "Tube". The carrier will move until tube 1 is exactly underneath the injector axis.

Select "**Rinse 1**", "**Rinse 2**" or "**Waste**". This will move the carrier to the tubes serving as rinse and waste station, respectively.

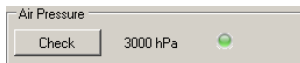
Click "**Air Cushion**", and select "**Slow**" or "**Fast**" movement. Use the arrow buttons to move the carrier stepwise in  $30\ \mu\text{m}$  steps (slow) or continuously (fast) to any desired position. The X and Y values show the actual position relative to the home position ( $X = 0$ ,  $Y = 0$ ).

The air cushion pressure is only present when the carrier receives a movement command from the software. Therefore, never touch the carrier or try to move the carrier manually when it performs software controlled movements. There is only one exception during alignment of the carrier to the grid of the table manually. For this purpose, switch on the air cushion pressure by clicking "Air Cushion" in "Manual Mode". In this mode it is possible to move the carrier safely by hand as long as the "Air Cushion" button is active. Please see chapter "Alignment of the Well Plate Carrier".



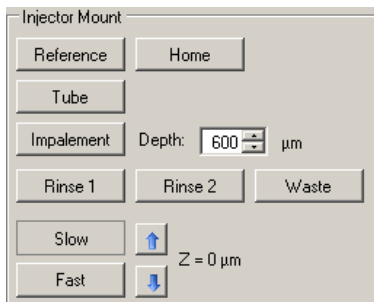
**Warning: Remove the injection needle before manually moving the carrier. Otherwise, there is the risk of severe injury and damage of the injector.**

## Air Pressure Section



Check the air pressure. It should be set to a minimum value of 3000 hPa, and should never exceed a value of 4 bar.

## Injector Mount = z-Axis Section



Click "Reference". The z-axis will perform its reference movement and will stop in home position. The depicted Z value is 0  $\mu\text{m}$ .  
 Click "Home" to move the z-axis into home position. The home position for the z-axis is defined as the position when the injection device is at its topmost position. The depicted Z value is 0  $\mu\text{m}$ .

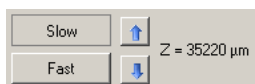
Click "Tube". The z-axis moves to a position from where a sample can be taken up from one of the reservoir tubes.



**Warning: Never try to move the z-axis to any tube position before moving the carrier to the respective tube. Otherwise, there is the risk of severe damage of the injector.**

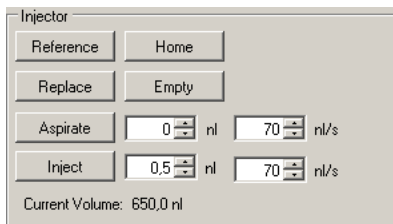
Click "Impalement". Select the "Depth" of impalement from the drop down menu.

Select "Rinse 1", "Rinse 2" or "Waste". The actual position of the z-axis will be displayed in "Z = x  $\mu\text{m}$ ".



Use the **arrow** buttons to move the z-axis stepwise in 20  $\mu\text{m}$  "Slow" or 200  $\mu\text{m}$  "Fast" steps up and down. The "Z" value shows the actual position of the z-axis in  $\mu\text{m}$  relative to the home position (Z = 0).

## Injector = Plunger Section



Click "Reference". The plunger will perform its reference movement and will stop in home position.

Click "Home" to move the injection plunger inside the z-axis into home position. The home position is defined as the topmost position.

Click "Replace" to start the needle installation or needle changing.

Click "Empty" to move the injector plunger continuously from the topmost position into the injection needle to extrude the oil filling of a new injection needle.


Click "Aspirate" to move the injector plunger upwards for aspirating the volume in nl selected from the up-down control beside the button. Be sure to immerse the tip of the needle into the medium you want to aspirate to avoid the suction of air into the needle.

**Note: You cannot aspirate more "volume" than it has been ejected before by the command "Empty".**



Click **"Inject"** to move the plunger stepwise from top to bottom, for injecting the volumes selected from the up-down control beside. It is possible to inject volumes from 1 to 100 nl.

## Changing the Injection Needle

Click on the icon  to open the **"Manual Mode"** dialog.


Use the injector section to test the functionality of the injector with installed injection needle to test the injector under more realistic conditions or to do injections manually. Click **"Home"** and wait until the plunger arrived at its topmost home position. Click **"Replace"** in "Injector Section" of "Manual Mode" if there is an used needle installed, and remove it carefully. Be sure not to bend the plunger wire.

Fill a fresh injection needle completely and air-bubble free with mineral oil. Install the needle by inserting the plunger wire end slowly into the capillary. Lock the luer connector tightly but not with excessive force.

## Alignment of the Well Plate Carrier

The well plate carrier hovers over the magnetic x/y table. For a smooth and proper movement, it is crucial that the table is kept dry and clean, and the carrier is properly aligned on the table.

Clean the x/y table with a soft tissue and pure alcohol.

Click on the icon  to open the **"Manual Mode"** dialog.

Activate the air cushion by clicking on the **"Air Cushion"** button. Now it is possible to move the carrier carefully by hand. Switch the Roboinject off for cleaning the x/y table and the carrier.

After cleaning, switch the Roboinject on. Activate the air cushion again to align the carrier precisely to the grid on the x/y table by hand. The air cushion will be automatically deactivated when you close the Manual Mode" dialog.

**Important:** A manual movement of the carrier is possible only if the Roboinject is switched on and the air cushion is present. Please make sure that the compressed air pressure is sufficient (minimum 3 bar).



**Warning:** Do not use aqueous solutions and / or cleaning agents for cleaning the magnetic x/y table. Otherwise, the carrier and / or the x/y table can be damaged. Please make always sure that the carrier is aligned to the grid of the table. Otherwise, a correct movement of the carrier is not possible.

Click **"Show Report"** icon .

Please see chapter **"Tools"**, "Show Report" of the main menu.

Click **"Clear Plate"** icon .

The **"Clear Plate"** button sets back all wells which are already assigned to a sample to the original, non-selected status. The deselected (crossed out) wells will remain.

Click **"Void Well"** icon .

The icon is for selecting or deselecting wells in the virtual well plate. If you activate the button **"Void Well"** you can select and deselect single wells or rows and columns. The deselected wells are crossed out and will not be injected.

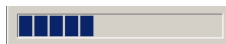
## Control View



The "Control View" on the right side of the main window of the Roboinject software is divided into two smaller windows: "Injection" and "Log" file.

In the "Injection" window the status LED indicates the status of the device. Green color indicates a valid connection between Roboinject and computer, red color indicates no connection.

The "Start" and "Break" buttons start and interrupt the injection process. The status bar below the buttons displays the injection proceeding during the injection run.



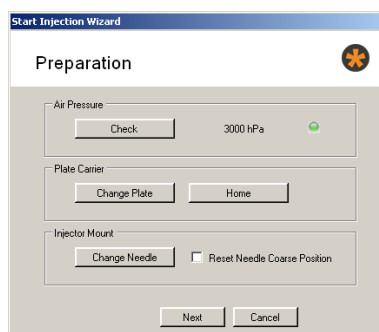
In the "Log" file window the injection process is documented.

## "Injection" Window

After completing the definition of the injection protocol the "Start Injection Wizard" proceeds with the final preparations for the injection.

Via "Start" and "Break" buttons you start and stop the injection manually. The status bar under the buttons will show the processing of the injection.

Click the "Start" button. The "Preparation" dialog of the "Start Injection Wizard" appears.



Air pressure: Please check the air pressure, the minimum has to be 3 bar = 3000 hPa. Check the connection status: A green LED indicates a sufficient air pressure.

**Plate Carrier:** Click "Change Plate". This command will move the carrier to an easily accessible position. Please remove the old well plate from the carrier, if necessary, Take a well plate from the type you specified in the "Plate Info" dialog box. Place the plate onto the carrier and press it against the springs in the lower middle and lower right corner of the carrier. Then put the rest of the plate down onto the carrier. The well plate position is correct when there is no play between the well plate bottom and the carrier surface at all edges and when the well plate has full contact to the left and upper side of the carrier holding frame.

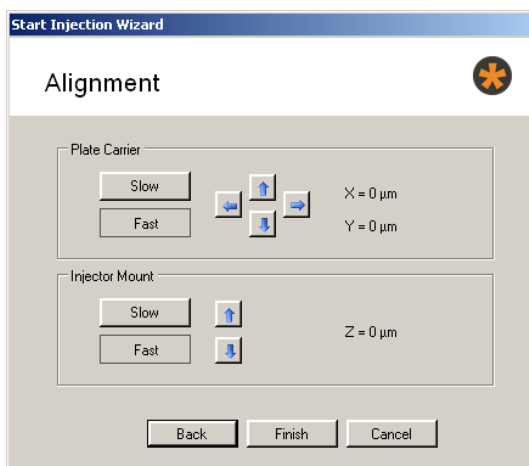
Place the reservoir tubes according to their appearance in the virtual well plate in the respective mounting holes. Do not use excessive force. Also place the rinse tubes and the waste tube at their designated positions.

Click "Home" for moving the well plate carrier into the home position.

**Injector Mount:** Please mount an injection needle. Click "Change Needle". Enable the check box "Reset Needle Coarse Position" to initialize the manual z-axis alignment after installation of the injection needle, if the needle length has changed.

Put the alignment device (with crosshairs) into well H12. Please read chapter "Alignment".

Click "Next". The "Alignment" dialog of the "Start Injection Wizard" appears.

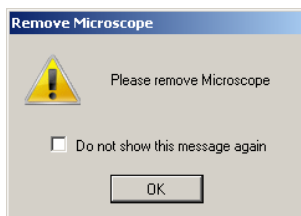


**Plate Carrier:** It is possible to move the plate carrier stepwise with the arrow buttons in x- and y- direction on the table. The actual position of the carrier in relation to the home position ( $X = 0 \mu\text{m}$ ,  $Y = 0 \mu\text{m}$ ) is displayed.

Use the arrow buttons to move the carrier stepwise in  $30 \mu\text{m}$  steps (slow) or continuously (fast) to any desired position. The X and Y values show the actual position relative to the home position ( $X = 0$ ,  $Y = 0$ ).

**Injector Mount:** It is possible to move the injector mount stepwise with the arrow buttons in z- direction on the plunger. The actual position of the carrier in relation to the home position ( $Z = 0 \mu\text{m}$ ) is displayed.

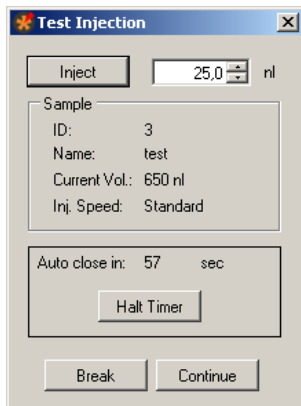
After proper alignment in x, y, and z- direction click "Finish". The "Remove Microscope" dialog appears.



Please enable the check box if you do not want to see this dialog again. You can display the dialog again when selecting the check box "Reset Remove Microscope dialog" in the "General" tab of the options dialog.

Click "OK" and a sequence of movements are performed. First, the carrier moves to "waste" position and the oil is extruded into the waste container. Then, the carrier moves to the first defined sample tube and the necessary volume is taken up. Finally, the test injection dialog opens if "Test Injection" was selected in the "Sample Information" dialog of the respective sample.

The "**Test Injection**" dialog appears immediately before starting the injection process of the selected sample.



This dialog gives the user the possibility to check the transmissibility of the tip opening of the injection needle. The defined sample volume will be injected into air. Before doing so, please place the microscope on the x/y table in a way that you can clearly see the needle tip.

Multi Channel Systems MCS GmbH recommends up to two test injections for checking the transmissibility of the tip opening. The additional volume for two test injections is aspirated automatically in addition to the injection volume necessary for all injections of the respective compound. The default test injection volume is half of the injection volume. If you have defined different injection volumes for a respective compound, it is half of the largest injection volume. If you do more than two test injections, the missing volume is automatically taken up during the injection process.

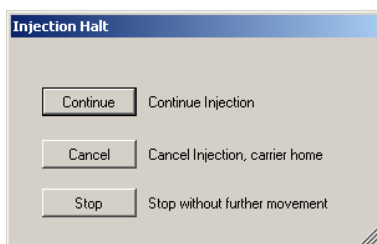
Click the button "**Inject**" to perform the test injection the volume of which is displayed in the up-down control window. The "**Sample**" window displays important sample parameters. In the "**Auto close**" window the time in seconds is counted down before this dialog is automatically closed.

Click "**Continue**" to start the injection process.

Click "**Halt Time**" if you need more time, for example, to check the tip opening by doing test injections.

Click "**Break**" to stop the test injection and the general injection process, for example, if the injection needle is broken or clogged.

The "**Injection Halt**" dialog appears.



To control the ongoing injection process you can choose between three possibilities:

Click "**Continue**" to continue with injection process.

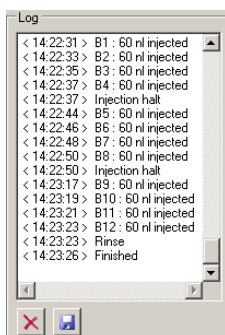
Click "**Cancel**" to stop the injection process. The carrier and the injection mount will automatically move to home position.

Click "**Stop**" to stop the injection process without any movement of carrier or z-axis. This is useful in case of severe malfunctions which could possibly lead to damages if further movements would be performed. After a successful injection process the Roboinject software displays the "**Injection Finished**" dialog.



Click "OK" to continue with the same well plate, or remove the plate and click "OK".

## "Log File" Window



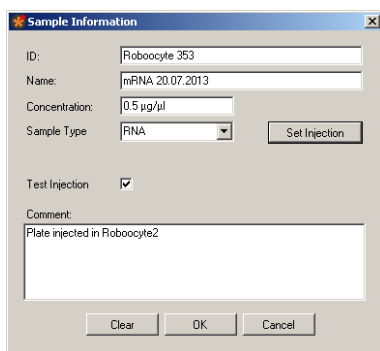
The log file is displayed in the "Log File" window. The "Log" file documents the injection process. To save the log file, click the "Save Log" icon. The log file has the extension "\*.log". To delete the log file, please click the icon "Clear Log" .. If you save the log file, the "Save As" dialog will open. Please browse through your folders, and define the directory you want to store the log file.

## Tube View



In the "Tube View" you control the definition and selection of the samples. You can use the eight reservoir tubes for eight different samples.

Click onto the symbol  of a "Edit Sample" icon.



The "Sample Information" dialog opens.

This dialog allows the definition of the respective injection samples inside of the 0.5 ml micro reaction tubes.

**Note:** The sequence of injection is always from left sample (No 1) to right sample (No 8). Please keep this in mind if the injection sequence of samples is of importance for you.

Enter an ID and / or a name which univocally identifies your sample in the "ID" and "Name" window.

Select the type of sample RNA, DNA or Other in "Sample Type", and its "Concentration".

**Important:** The minimal volume which always has to remain in the tube reservoir is 1  $\mu$ l. For example, if you need 4  $\mu$ l sample volume for your injection run, please make sure that you start with at least 5  $\mu$ l. This is because the injection needle keeps always a safe distance to the tube bottom to avoid uptake of contaminants and clogging.

Enable the check box "Test Injection". The "Test Injection" dialog will appear before the injection process of a sample is started. Please see chapter "Test Injection" dialog.

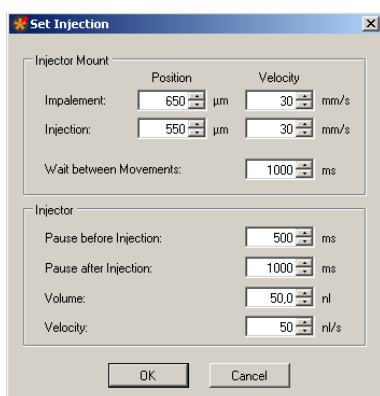
Type notes concerning the actual experiment into the "Comment" text box, for example, the type of the sample when selecting "Others". The comments are saved in the "Plate Info" file and shown in the report sheet.

Click the "**Set Injection**" button for setting injection parameters for that sample. Ignore the button for keeping the default settings. Click "**OK**" to complete the settings for this sample. Click "**Clear**" if you want to restart with default values, and "**Cancel**" if you want to go back to the main window without changes.

Repeat the settings in "Sample Information" of other tubes until all samples you want to inject are defined.

**Note:** The sequence of injection is always from left sample (No 1) to right sample (No 8). Please keep this in mind if the injection sequence of samples is important for your injection process.

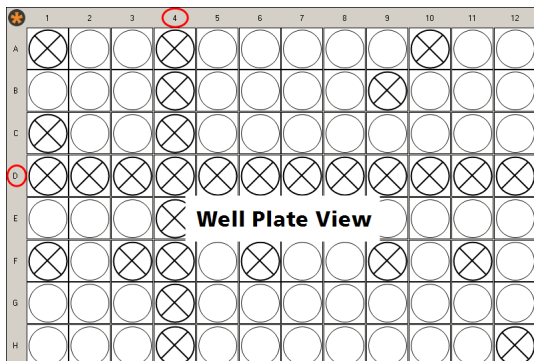
Click "**Set Injection**". The "**Set Injection**" dialog opens. The parameters are also available in the "Inject" tab of the "Option" dialog in main menu "Settings". Please read chapter "Inject" tab of the "Options" dialog in "Settings" menu.




After finishing the settings in the "Sample Information" dialog the tube icons appear colored, according to their content for a good discrimination between the compounds.




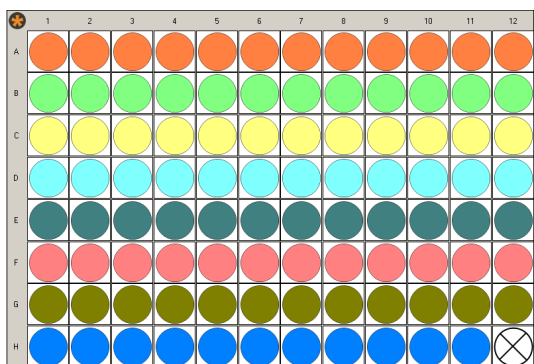
## Well Plate View



This prominent "Well Plate View" shows a 96 well plate. When using a 384 well plate, this view is automatically adapted.


Click the "Void Well"  icon . To select or deselect a single well, please click onto the respective well. To select or deselect complete rows or columns, click onto a letter on the left side to mark a horizontal row, or onto a digit on top to mark a column. Deselected wells are crossed out, and will not be injected. The well H12 is deselected by default. It is used for the alignment tool, please read chapter "Alignment" for more information.

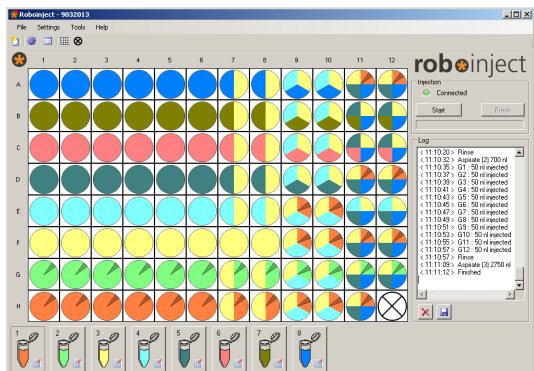
Click the "Clear Plate"  icon to set back all wells to the original, non-selected status. The deselected (crossed out) wells will remain.



## Defining the Samples

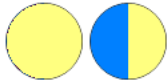
After setting up the "Sample Information" dialog, the samples of the tubes are fixed. To define which well should be injected with which sample, please click onto the tube icon first, and then onto the well icon. The color of the well will immediately change into the symbol color of the sample of the selected tube. Choose another tube to assign one or several wells to that tube sample respectively. It is possible to assign any well to any sample. Use the letters and digits to correlate horizontal rows and vertical columns to the samples.

Use the Roboinject icon  to select and deselect the sample of the complete well plate.



## Defining the Number of Injections into a single Oocyte

In some experiments it is necessary to inject more than one compound into a single oocyte. The Roboinject program gives the possibility for up to four injections into the same oocyte. To define the well for several injections, please click onto the tube icon first, and after that onto the well. The color of the well will immediately change into the symbolic color of the sample of the selected tube.



Select a second tube icon and click onto the same well again. One half of the color of the well will immediately change into the color of the second sample.



Select a third tube icon and click onto the same well again. One third of the color of the well will immediately change into the color of the third sample. Select a fourth tube icon and click onto the same well again. One quarter of the color of the well will immediately change into the color of the fourth sample.

Click "Clear Plate" to set back all wells to the origin, non-colored status. The deselected (crossed out) wells will remain. After successful injection the wells (or sections of the well) will be marked with an arrow.



## INJECTION

### About Injection with the Roboinject

Manual injection is often a limiting factor in high-throughput screens. The Roboinject injects *Xenopus* oocytes and other large cells or small embryos in a fully automated way.

For example, injection and cultivation of *Xenopus* oocytes are performed using disposable standard 96 well plates, which are commercially available from several providers. The oocytes are plated into the wells in a couple of minutes and can be kept there for several days. They quickly settle within the cone-shaped wells and adhere to the well bottom after a few hours. The oocytes do not have to leave the plate anymore; you can easily transfer the oocytes from the incubator to the Roboinject and back again. For other cells or organisms custom-designed well plates can be easily adapted.

The cDNA, mRNA or compound to be injected is automatically taken up into the injection needle and then pushed into either the nucleus or the cytoplasm by the positive displacement method. Using positive displacement has the advantage that the injected volume is always as pre-selected. Importantly, it is independent of the viscosity of the samples or the opening diameter of the injection needle. Another advantage of positive displacement based injectors when compared to air-pressure driven system is, that there is no need of tedious volume calibrations.


The overall expression rate you can achieve with the Roboinject is by no means worse than success rate with manual RNA injection of *Xenopus* oocytes and can reach close to 100 percent. Of course, the success rate depends on the receptor type and oocyte performance.

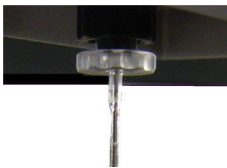
### Strategies for a Successful Injection

Multi Channel Systems provides injection pipettes with an inner diameter exactly matching the plunger wire. The provided injection pipettes are glued to luer-fittings and therefore ready to use. If you nevertheless decide to fabricate your own injection pipettes you should keep in mind that you can only use the capillaries listed in the chapter "Ordering Information" in the Appendix. Other capillaries will not work properly.

The Roboinject software allows to define the impalement and injection position for each sample separately during the well selection process. The "Impalement Position" and the "Injection Position" in the main "Set Injection" window denotes the distance of the needle tip to the well bottom (zero value). This means, for example, that for an oocyte with a diameter of 1.2 mm an impalement or injection position of 650  $\mu\text{m}$  would correspond to an oocyte impalement depth of 550  $\mu\text{m}$  ( $1200 \mu\text{m} - 650 \mu\text{m} = 550$ ) Please read chapter "Inject" tab of "Option" dialog in "Settings" menu.

### Changing and loading the Injection Needle

Click to start  the "Manual Mode" dialog. Use the "Change Needle" command to change or install a new needle. "Change Needle" will move the plunger and carrier in home position and move the z-axis a bit down for better access to the luer connector for the needle.



Use a fine pipette tip, for example, a microloader tip filled with mineral oil. Insert the fine tip into the injection needle as close as possible to the needle tip. Fill the injection needle completely and air-bubble free with oil. The remaining air in the tip region will be cleared automatically later on.



Insert the plunger wire carefully into the needle end, move it slowly up and close the luer lock. Part of the oil will drop out of the needle tip. Remove it with a soft tissue from the table. Be careful not to break the glass capillary.

## ***Xenopus* oocytes**

The impalement and injection distances for cDNA are preset to 300  $\mu\text{m}$  and 550  $\mu\text{m}$ , respectively. (From the bottom of the well = 0 value).

These distances have been shown as the optimum values for DNA injection. The overall expression rate you can achieve by this method is 80 percent of the oocytes. You can define your own impalement and injection depth, but MCS recommends using the default values in order to achieve the best expression rate.

You may wonder why the injection needle is placed so deeply for cDNA injections while the nucleus is known to be located near the animal pole. As it seems, the nucleus is pressed down by the injection needle until the pressure is sufficient for the needle to penetrate the membrane of the nucleus.

Messenger RNA is injected into the cytoplasm. Generally, it is not important where in the cytoplasm the RNA is released. The default impalement and injection distances for mRNA are 650  $\mu\text{m}$  and 550  $\mu\text{m}$ , respectively. These are safe values even for smaller oocytes.

## **Running a test for nuclear injection with a dye**

To demonstrate the precision of the injection and to practice the general handling, you may consider running a test injection with the dye "Trypan Blue", which stains proteins unspecifically, before you start to inject your constructs. Injected oocytes are then torn open with forceps, thus releasing the complete, stained nucleus.

1. Dissolve Trypan Blue in distilled water. The solution should appear bright blue.
2. Remove any particles with a 0.22  $\mu\text{m}$  syringe filter or something similar.

Continue with the next chapter "Preparations for Injection". You can use this dye solution to mimic cDNA. If you have successfully performed the test or if you prefer to start your first experiment under real conditions, you can right away start to use your cDNA or mRNA. You may consider the test injection again if you observe any troubles.

The following pictures show injected oocytes. In the left picture, the penetration site of the needle is clearly visible in the middle of the animal pole. The right picture shows an opened oocyte. Only the nucleus is stained, showing that the nucleic membrane is still intact and therefore impermeable for the dye.



## **Preparations for Injection**

Generally, you can use cDNA or mRNA concentrations and injection volumes as described in the literature or as applicable for your experiment. In the following, typical cDNA or mRNA concentrations and recommended treatment of the sample are described. Please follow this advice if you are new in operating the Roboinject or if you have observed trouble with the injection process.

You will also need the provided stereo microscope and alignment device (with crosshairs) for the alignment of the injection needle. Please read chapter "Alignment".

**Note:** The cDNA or mRNA sample has to be very clean and pure to obtain good results. Any particles, dirt, dust, and so on could clog the needle. Please check the sample for protein contaminations, cellular debris, residues of the extraction kit, or a high salt content.

## **Checking the Oocyte Quality**

You need oocytes plated in standard 96 well plates. You should only use oocytes of a very high quality and of uniform size. Make sure that all oocytes are positioned with the animal pole (dark side) up, when you want to inject cDNA into the nucleus. The oocytes will have adhered to the well bottom after about 2 to 3 hours. Do not use the cells before. Best results have been obtained if oocytes have been incubated over night before use. See also "Preparation of *Xenopus* Oocytes" in the Appendix.

## cDNA / mRNA Concentration and Storage

- Depending on the efficiency of protein expression, about 10 to 50 ng of cDNA (about 250 to 2500 ng of mRNA) is sufficient to maximally express the desired receptor in all 96 oocytes. Much lower amounts might be appropriate if you need a reduced expression level, for example for electrophysiological recording of ion channels.
- A typical cDNA sample has a concentration of 10 to 50 ng/ $\mu$ l, a typical mRNA sample has a concentration of about 50 to 500 ng/ $\mu$ l.
- DNA or RNA should be stored in sterile, nuclease-free 0.5 ml or 1.5 ml Eppendorf tubes (or similar reaction tubes) at  $-80^{\circ}\text{C}$ .
- Highly concentrated stock solutions should be diluted with nuclease-free water before use. Do not use saline solutions because salt crystals may form during injection and may cause the needle to clog.

## Preparing the Sample for Injection


1. Take the sample out of the freezer at least 10 minutes before the injection.
2. Let it stand at room temperature until the sample is completely thawed.
3. Mix the sample thoroughly, for example, by vortexing for a short moment, or manually by striking the side of the tube with a finger.
4. Spin the sample in an Eppendorf centrifuge (or a similar centrifuge) at full speed ( $>10000\text{ g}$ ) for 5 minutes immediately before the injection for sedimentation of any remaining particles that might clog the needle.

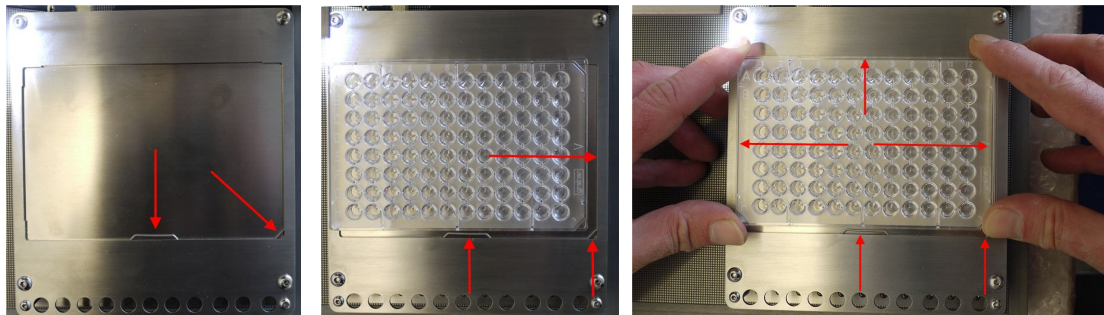
## Filling and Placing of the Reaction Tubes

3. Please use the provided reaction tubes as sample reservoirs.
4. Fill the respective reservoir tubes (position 1 to 8) always with a sample volume that is  $1\ \mu\text{l}$  more than needed for the injection of all selected oocytes. The needle tip will not go down to the very well bottom but will always stop at a safety distance. This avoids breakage and contamination of the tip.
5. Fill the two rinsing reservoirs (position 9 and 10) with at least  $200\ \mu\text{l}$  of nucleotidase-free water.
6. The waste reservoir (position 11) can be kept empty, it will be used for oil removal only.
7. Place the tubes centered into the mounting holes. Bend the tube caps in a way that they are perpendicular to the tube. The tube caps should be positioned that they are facing away from the plate.

## Setting Up the Well Plate

### Inserting or Changing the Well Plate


Click the icon  to open the "Manual Mode" dialog. Select "Change Plate" in the "Global" section to move the carrier into home position. Remove the used well plate if necessary. Place the type of well plate which is defined in the "Plate Info" dialog in correct orientation on the carrier.

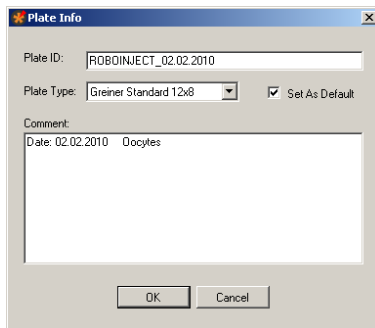


Place the well plate onto the carrier and press its side against the springs in the lower middle and lower right corner of the carrier. Then put the rest of the plate down onto the carrier. The well plate position is correct when there is no play between the well plate bottom and the carrier surface at all edges and when the well plate has full contact to the left and upper side of the carrier holding frame.

**Important: The well plate should be immovably fixed on the carrier, otherwise the injection will not be successful!**

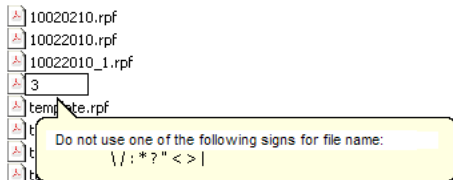
## Creating a new File

1. Click "New File" icon  either on the toolbar or on the "File" menu. The "Plate Info" dialog box opens.



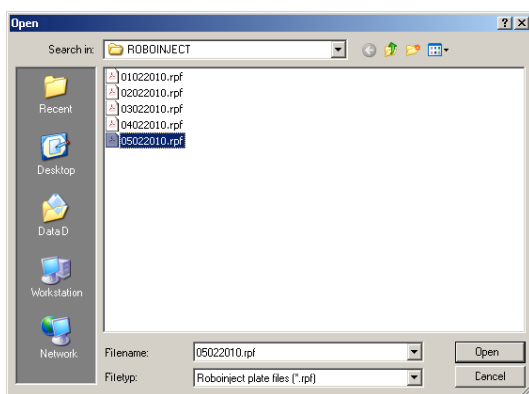
2. Type in the "Plate ID", select the "Plate Type", and all information ("Comment") you want to associate with this well plate, for example the oocyte batch, name of the operator, and so on.
3. Click "OK" when you are finished. The "Save As" dialog box is displayed. The plate ID is also the suggested file name.
4. When opening the directory, you can choose another file and / or rename the file, if necessary. Right-click on the file name, and choose "Rename". All characters are allowed in the file name, except the following: slash mark (/), backslash (\), colon (:), asterisk (\*), question mark (?), quotation mark ("), less than (<), greater than (>), and vertical bar (|).

If you try to use one of these characters the following error message appears



## Opening a previously saved File


1. If you want to inject the same plate again or review previously injected cells, open the appropriate well plate file.
2. Click "Open" on the "File" menu. The "Open" dialog box appears.



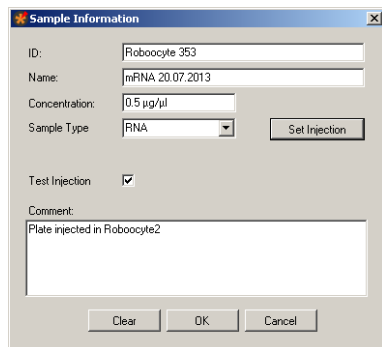
3. Select the Roboinject file (\*.rpf) you want to open.
4. Click "Open". The well plate window now shows the status of the already saved plate; and the Roboinject header shows the name of the actually opened file.



## Defining the Injection Samples

1. The next step after creating a new file and well plate is the definition of the respective injection samples inside of the 0.5 ml micro reaction tubes.
2. Click on the symbol  of one of the sample reservoir tubes to open the "Sample Information" dialog box. Please read also chapter "Sample Information" dialog.

**Note:** The sequence of injection is always from left sample to right sample. Please keep this in mind if the injection sequence of samples is of importance for you.



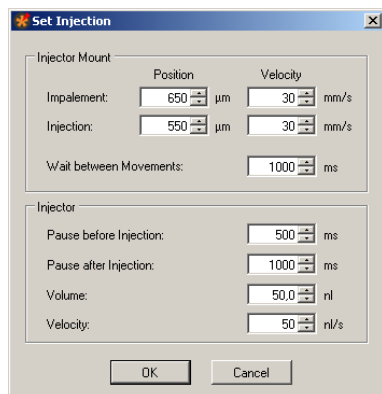
The "Sample Information" dialog box contains the following fields and controls:

- ID: Robocyte 353
- Name: mRNA 20.07.2013
- Concentration: 0.5 µg/µl
- Sample Type: RNA (dropdown menu)
- Test Injection:
- Comment: Plate injected in Robocyte2
- Buttons: Clear, OK, Cancel, Set Injection

Enter an ID and / or a name which univocal identifies your sample. Select the type of "Sample" and its "Concentration".

**Note:** The minimal volume which always has to remain in the tube reservoir is 1 µl. For example, if you need 4 µl sample volume for your injection run, please make sure that you start with at least 5 µl. This is because the injection needle keeps always a safety distance to the tube bottom to avoid uptake of contaminants and plugging.

Click the button "Set Injection" for changing injection parameters. The "Set Injection" dialog opens. Please read also chapter "Inject" tab of "Options" dialog in main menu "Settings".



The "Set Injection" dialog box contains the following settings:

- Injector Mount:
  - Impalement: 650 µm, 30 mm/s
  - Injection: 550 µm, 30 mm/s
  - Wait between Movements: 1000 ms
- Injector:
  - Pause before Injection: 500 ms
  - Pause after Injection: 1000 ms
  - Volume: 50.0 nl
  - Velocity: 50 nl/s
- Buttons: OK, Cancel

Ignore the "Set Injection" button for keeping the default settings.

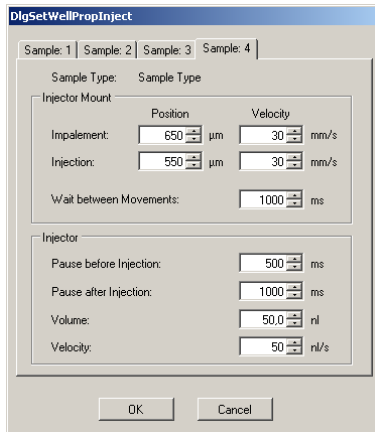
Enable the check box "Test Injection" if you want to use this feature. Write a comment in the "Comment" text box.

Click "OK" if you finished the settings of the sample, "Clear" if you want to restart with default values and "Cancel" if you want to go back to the main window without changes.

Repeat the steps above until all samples you want to inject are defined.

## Adjusting the Injection Parameters for each Well separate


It is possible to adjust the settings for the injection for each single well separate. Click with the right mouse button onto a well in the virtual well plate, and the following dialog appears. Now you can adjust the injection parameters for this specific well.




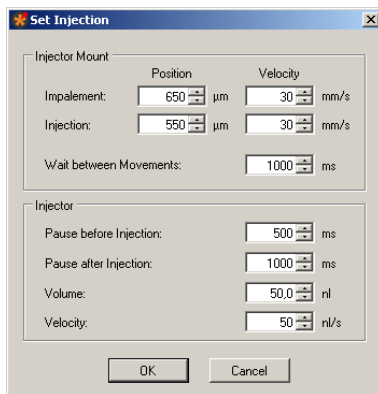
If the oocyte in the specific well should be injected with different samples, you can adjust the injection parameters for each sample separately in the respective "Sample" tabs.

## Selecting Oocytes for Injection

The next step after definition of the injection samples is the allocation of the sample(s) to the respective wells.


Click the "Void Well" icon  to exclude certain wells from injection. The deselected wells will be crossed out.

Click the "Clear" icon  to set back the virtual well plate to the origin, non-selected status. First, please select an injection sample.



To assign a sample to a well, please click onto the colored tube on the icon first, and after that on the well, you want to assign to the sample. The color of the well immediately changes to the symbol color of the sample tube.

Click on the row letters A to H to assign single rows or on the column numbers 1 to 12 to assign single columns.

If you want to select all wells, click on the Roboinject icon  on the upper left corner of the virtual well plate.

If you like to apply a second sample, please proceed with the next sample. Again, the wells change their color to the sample color when you select them. If you select wells already selected for injection with a different sample the well will be divided into two halves with different colors. Theoretically you can inject a single cell with four different compounds ending up with a virtual well split into four quarters with the four different sample colors.

## Changing the Well Plate and Loading the Needle

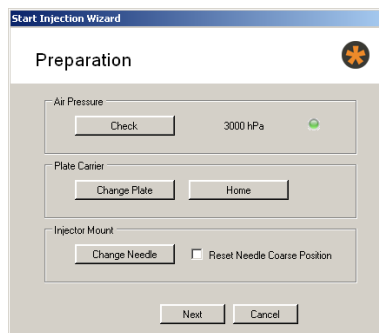
You will need the cDNA or mRNA samples, the well plate loaded with oocytes, and an injection needle. You can reuse injection needles several times as long as they are not clogged or damaged. You will also need the provided stereo microscope and the provided alignment device (with crosshairs) in order to position the well plate horizontally and vertically as well as the injection needle before you start the injection. The alignment wizard will lead you through all necessary steps.

**Note:** Make sure that all oocytes have had time to settle for several hours and are positioned in the well with the animal pole (dark side) up. See also "Preparation of *Xenopus* Oocytes" in the Appendix.



**Warning:** Do not move the z-axis by hand while installing the injection needle. In all cases, perform an alignment after you have replaced the needle or the well plate.

1. Click the "Start" button in the main window. The "Start Injection Wizard", "Preparation" appears.



2. Click "Change Plate" in "Plate Carrier" window. After loading or replacing the well plate that, please click "Home". The carrier moves to home position (X = 0, Y = 0).
3. If necessary, remove the old well plate from the carrier.
4. Take a well plate from the type you specified in the "Plate Info" dialog. Place it onto the carrier and press its side against the spring in the lower right corner of the carrier. Then put the rest of the plate down onto the carrier. It should fit tightly and immovably now.
5. Put the alignment device (with crosshairs) in well H12.
6. Enable or deselect the check box "Reset Needle to Coarse Position".
7. Click "Change Needle" in "Injector Mount" window.

## Changing or loading the Injection Needle

1. Use the "Change Needle" command to change or install a new needle. "Change Needle" will move the plunger and carrier in home position and move the z-axis a bit down for better access to the luer connector for the needle.
2. Use a fine pipette tip, for example, a micro loader filled with mineral oil. Insert the fine tip into the needle as close as possible to the needle tip. Fill the needle completely and air-bubble free with oil. The remaining air in the tip region will be cleared automatically later on.
3. Insert the plunger wire carefully into the needle end, move the needle slowly up and close the luer lock. Part of the oil will drop out of the needle tip. Remove it with a soft tissue from the table. Be careful not to break the glass capillary.

## Reset Needle to coarse Position

Enable the check box "Reset Needle Coarse Position" if the needle length has changed since the last alignment.

During the alignment process, the injector mount (z-axis) is moved automatically to the last alignment position minus a safety distance of 3 mm (so called "Coarse Position"). When using a needle that is longer than the one used last time, there is a risk that the needle will break. You should reset the memorized "Coarse Position" in this case. The z-axis will not move down during the automatic coarse positioning and the needle will be safe.

Click the "Next" button for displaying the "Alignment" dialog of the "Start Injection Wizard".

## Alignment

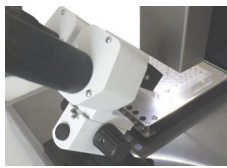
You have to adjust the position of the carrier and the needle under microscopic control. The Roboinject always moves relative to a standard = home position. The aim of the alignment process is to define this standard position. If this is not done properly, the Roboinject moves to wrong positions. The alignment process also compensates for minor changes in the dimensions of different well plates and variations in the length of the needle. To achieve a high expression rate, it is essential to perform an alignment each time before you start an injection sequence.

**Note:** The alignment is very important for obtaining best results. An alignment has to be performed each time before you start an injection. It is very important that the glass capillary is accurately centered on the alignment device. Otherwise, the oocytes will not be properly impaled and injected.

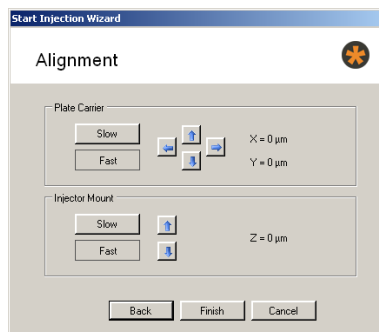


**Warning:** Hitting the alignment device can cause the injection needle to shatter, possibly projecting splinters, which can be dangerous. Eye protection should be used, and this alignment should never be observed up close with an unprotected eye.

Place the microscope in a position onto the x/y table which allows to focus on the alignment device plugged in well H12.



Open the "Alignment" dialog in the "Start Injection Wizard".



1. Switch in "Plate Carrier" to "Fast". The movement of the z-axis and the carrier commanded by the software controls will now be fast.
2. Move the injector mount (z-axis) and the carrier by pressing the arrow keys on the alignment dialog until the needle is almost centered on the alignment device.
3. Switch to "Slow". The movement will now be carried out in small single steps.
4. Fine-tune the position until the alignment device is almost touched by the tip of the needle. If you do this the first time, you may have difficulties seeing the needle three-dimensionally. The needle may appear to be centered correctly, but instead the needle is still a way above the alignment device and shifted to the front. If you do not feel sure about this, lower the needle slowly and carefully.

**Important:** Do not touch the alignment device with the injection needle! The fine tip should blunt and will not impale the oocyte or the needle will break.

5. However, you will see that after a bit of experience, the alignment is performed very quickly and without trouble. If you need to start the alignment again, for example, if you have moved the carrier accidentally by hand, click "Back" to go back to the "Preparation" dialog, and "Next" to restart the alignment process from step 1.

### Adjustment position (Injection)





6. Click "**Finish**" after successful alignment.

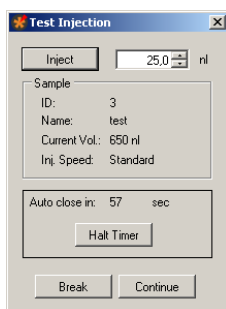
7. The current position is saved, and the old alignment position is overwritten. All following movements relate to the new standard position now.



**Warning:** Do not click "**Next**" if the alignment has not been finished. This could lead to wrong movements of the Roboinject, which are potentially hazardous. If you want to cancel the alignment, leave this dialog box by clicking "**Cancel**" or "**Back**".

## Starting the Injection

To start the injection after successful alignment, simply click the "Finish" button in the Alignment dialog window. After the oil has been pushed out to the waste tube and the volume of the first sample has been aspirated, the "Test Injection" dialog appears if activated.



This dialog gives the user the possibility to check the transmissibility of the tip opening of the injection needle. The respective sample volume will be injected into air. Before doing so, please place the microscope on the x/y table in a way that you can clearly see the needle tip.

Multi Channel Systems MCS GmbH recommends up to two test injections for checking the transmissibility of the tip opening. The volume of compound needed for this feature is aspirated in addition to the injection volume, defined in "Injection" tab of "Options" dialog in menu "Settings". You define, for example, 50 nl injection volume. Then 50 nl are additionally aspirated, sufficient for two test injections with 25 nl each. If you do more than two test injections, the missing volume is automatically taken up during the injection process.

Click the button "**Inject**" to inject the test volume of compound in nl which is displayed in the up-down control window.

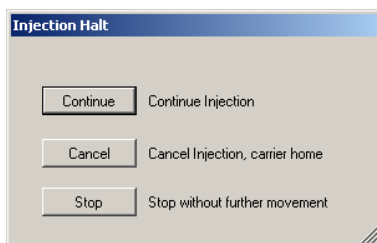
The "**Sample**" window displays important sample parameters. In the "**Auto close**" window the time in seconds is counted down before this dialog is automatically closed.

Click "**Continue**" to start the injection process.

Click "**Halt Time**" if you need more time, for example, to check the tip opening by doing test injections.

The "**Injection Halt**" dialog appears.

Click "**Break**" to stop the test injection and the general injection process, for example, if the injection needle is broken or clogged. To control the ongoing injection process, you have three possibilities:



Click "**Continue**" to continue with injection process.

Click "**Cancel**" to stop the injection process. The carrier and the injection mount will automatically move to home position.

Click "**Stop**" to stop the injection process without any movement of carrier or z-axis. This is useful in case of severe malfunctions which could possibly lead to damages if further movements would be performed.

## Saving the Well Plate

After a successful injection process of all samples the Roboinject software displays the "Injection Finished" dialog.



Click "**OK**" to continue with the same well plate or remove the plate and click "OK".

The "Plate Info" file including the well plate status, file info, and comments is saved automatically to the destination you have specified in main menu "Settings", "Directories".

## TROUBLESHOOTING

### Troubleshooting

Most problems occur seldom and only under specific circumstances. In most cases, it is only a minor problem that can be easily avoided or solved.

If the problem persists, please contact your local retailer. The highly qualified staff will be glad to help you. Please inform your local retailer as well, if other problems that are not mentioned in this documentation occur, even if you have solved the problem on your own. This helps other users, and it helps MCS to optimize the instrument and the documentation.

Please pay attention to the safety and service information (chapter "Important Safety Advice". Multi Channel Systems has put all effort into making the product fully stable and reliable, but like all high-performance products, it has to be handled with care.

### Clogged Injection Needle

If you observe that your injection needles are frequently blocked, please check the blocked needle under a microscope to find the cause of the problem.

#### Injection Needle is blocked from Outside

DNA / RNA solution flows out of the tip and dries out. The sticky residues will block the tip.

Remove the blockage by moving the needle into fluid for a short moment to dissolve salt crystals, for example you can move it into the alignment well filled with distilled water (without alignment tool). If this does not help, you can move the needle right down onto the alignment device so that the needle actually touches the alignment device. You may also try to cut a little piece from the tip with a sharp blade.

Possible causes:

? The time lag between alignment, setting the pressure and starting the injection can be too long. This is especially likely if you are a fresh user and need too much time for the alignment. You should not need more than about three minutes for the alignment and starting the injection. Practice the alignment process and the pressure settings with a typical solution without actually injecting to get used to the procedure.

#### Injection needle is blocked from Inside

Particles in the RNA / DNA sample can clog the needle. The vertical alignment of the needle makes clogging more likely if particles are present than the angular direction of the needle in a manual injection setup.

Possible causes:

? Can you see particles under the microscope? There may be residues from the DNA / RNA preparation, for example PCR beads, particles from a column, remains of an agarose gel or extraction kit, and so on. There may also be cell debris, if the DNA is extracted from bacteria.

? If you cannot see any possible cause, check your RNA / DNA solution. Maybe the concentration is too high, or there is too much salt, too much protein, or too much cell debris in the preparation?

Centrifuge the sample longer or more intensely (for example for 5 min at 2000 g or for 1 min at 6000 g) to remove any particles immediately before loading the needle. Take up the solution carefully from the top, not touching the well bottom with the pipette tip.

Prepare the RNA / DNA sample more carefully next time and control the purity according to standard molecular biology protocols.

? The pipette tips (Microloader) you are using to fill the injection needle with oil may be contaminated. For example, if the tip rack has been opened for too long, the tips can get dusty.

Use a rack with new clean pipette tips.

? The injection needles may be contaminated. If the unit has been opened for too long, dust can deposit in and on the needles. Use a new unit of injection needles.

## **Injection Needle gets blocked during Injection**

If the injection needle is fine when you start the injection, but gets clogged during the injection sequence, impurities in the DNA / RNA sample can still be the cause, because the particles may sink down slowly. Check the possible causes for the problem "Injection needle is blocked from inside" as well as the following.

Possible causes:

? Contaminations in the cDNA / mRNA sample slowly sink down during injection and cause the needle to clog.

Inject a well plate without oocytes, for example a well plate filled with mineral oil. Check the injected volume with a microscope. If the problem persists, the cDNA / mRNA sample itself causes the trouble. Please see preceding paragraphs "Injection needle is blocked from inside" for further advice.

## **No or Low Expression**

You should expect an expression rate of close to 100 percent for mRNA injection and a success rate of up to 70 percent when injecting cDNA, depending on the receptor and the oocytes, of course.

Please make sure that the DNA / RNA sample you use has been sufficiently tested and has a good expression performance. Make sure that the oocytes show a good general performance as well.

## **Oocytes are not hit during Injection**

If the injection needle does not clog, but the oocytes do not show a high expression rate, you should check whether the oocytes are hit during injection. Please perform a test injection with the dye Trypan Blue to analyze the situation further (see "Strategies for a Successful Injection" in the "cDNA / mRNA Injection" section).

Possible causes:

? The wrong well plate type has been used.

Make sure you use a well plate of the type that you defined in the General Options dialog box. Check the "Sources of Supply" if you are not sure about the recommended product.

? The well plates are warped.

Well plates have to be very flat to make sure that the oocytes are centered and hit properly. Check each plate before use. Do not use warped or sterile-packed plates (as they are known to have problems with warping due to the sterilization process). Do not autoclave or sterilize plates by heat.

? The alignment has not been performed properly.

Repeat the alignment and make sure that you see the needle clearly and three-dimensionally under the microscope. The fine tip of the needle should be positioned inside the groove in the middle of the crosshairs. Practice the alignment process without actually injecting to get used to the procedure. Let another person aid you if you are not sure whether the tip of the needle is really centered on the alignment device.

? If the alignment is fine, the impalement and injection depth have to be optimized. Generally, the default values (300 / 550  $\mu\text{m}$  for cDNA and 650 / 550  $\mu\text{m}$  for mRNA) are fine for all oocytes. But you may want to optimize them if your expression rate stays consistently below 80 percent, even for a good receptor.

Modify the injection distance values until you successfully hit the oocytes. Start with the standard values (300  $\mu\text{m}$  for DNA and 650  $\mu\text{m}$  for RNA) and lower it in steps of 50  $\mu\text{m}$ .

## **Error Messages**

The alignment was not properly performed. Please repeat the alignment procedure.

The plate file is not compatible.

If you rename a plate file, please do not use the following signs.

## APPENDIX

### Technical Support

Please read the "Troubleshooting" part of the manual or help first. Most problems are caused by minor handling errors. Contact your local retailer immediately if the cause of the trouble remains unclear. Please understand that information on your hardware and software configuration is necessary to analyze and finally solve the problem you encounter.

If you have any question or if any problem occurs that is not mentioned in this documentation, please contact your local retailer. The highly qualified staff will be glad to help you.

Please keep information on the following at hand

- Description of the error (the error message text or any other useful information) and of the context in which the error occurred. The more information on the actual situation you can provide, the easier it is to track the problem.
- The serial number of the device. You will find it at the rear side of the injector.
- The software and hardware version you are currently using. On the "Help" menu, click "About". The displayed dialog box shows the version numbers.
- The operating system and service pack number on the connected computer.
- The hardware configuration (microprocessor, frequency, main memory, hard disk) of the connected computer. This information is especially important if you have modified the computer or installed new hard- or software recently.

## MAINTENANCE

### Cleaning of the System

Always unplug the Roboinject prior to cleaning. To clean the exterior surfaces, use a lint-free cloth to remove loose dust. For more efficient cleaning, use a soft cloth dampened (not soaked) with an aqueous solution of 75 % isopropyl alcohol, or a mild detergent.

### Service and Maintenance

Please make sure that you read this chapter thoroughly. Proper handling and cleaning of the Roboinject will ensure reliable and long life time of the equipment. Most often, troubles are simply due to mishandling. See also chapter "Troubleshooting".

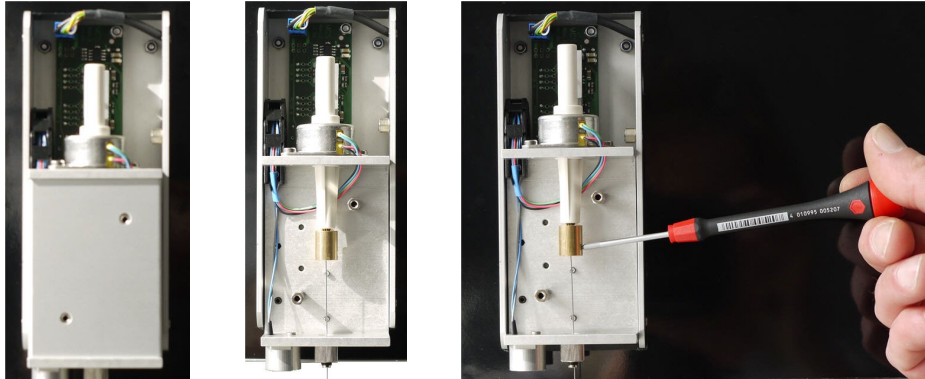
### Cleaning the x/y Table

You prevent a malfunction of the carrier by cleaning the x/y table regularly. Use a soft tissue and pure alcohol (100 %) to clean the x/y table.

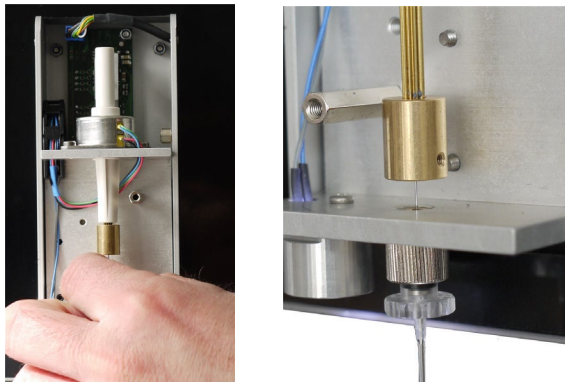


**Warning:** Water may provoke a corrosion of the carrier. Residues of cleaning agents may interfere with the function of the carrier or damage the surface of the x/y table. Do not use an aqueous solution or cleaning agents.

## Replacing the Plunger



To replace the plunger inside the injector mount, please remove the cover of the injector mount by pulling it into upward forward direction. Unscrew the screws on the second lid of the injector mount, and remove the smaller cover. First, open one of the screws of the plunger brackets only. Use the allen key from the accessory package. Pull out the old plunger. If the plunger does not come out easily, also loosen the second screw. Disconnect the plunger and move it downward through the hole at the bottom of the injector mount which leads to the luer lock for the injection needle.



Insert a new plunger on the same way. Insert it into the plunger bracket. Tighten the screws. The two screws in the plunger bracket squeeze the plunger between each other, and allow to adjust the plunger. The plunger should be aligned without tension between the plunger brackets and the hole at the bottom of the injector mount which leads to the luer lock for the injection needle. You can correct the alignment by loosen one screw and tighten the other.

## About Preparation of *Xenopus* Oocytes

The materials and methods described in the following are kindly provided by the Bayer AG, Leverkusen. The described procedures have been optimized over several years for best performance of the oocytes and highest throughput. We recommend that you follow the instructions for obtaining best results. Refer to standard protocols if you need more detailed information on the subject.



**Warning:** Only qualified personnel should be allowed to perform laboratory work. Always make sure you fulfill the requirements of local regulations and laws. Work according to good laboratory practice to obtain best results and to minimize risks.

## Rubber ball for oocyte transfer pipette

- Glass tool for moving oocytes: Glass Pasteur pipette, sealed over a Bunsen burner, and melted into a golf-club like shape.
- Petri or cell culture dishes, 100 mm
- Petri dishes, 60 mm
- Beaker, 100 ml
- Razor blade
- Forceps
- Parafilm
- Standard laboratory equipment

## Chemicals

### Collagenase

For defolliculation:

- Fresh 1.52 mg/ml collagenase in Barth's solution without Calcium. The concentration has to be optimized according to the collagenase batch and experimental conditions, see also chapter "Defolliculation". Do not prepare solutions in advance as collagenase activity may decrease rapidly even if the solution is stored at -20 °C.
- Collagenase from *Cl. histolyticum* ca. 0.17 to 0.28 U/mg (according to Wunsch, 1963) lyophilized.

### Gentamicin

- Stock solution: 50 µg/ml gentamicin (free base) in Barth's solution. 1 ml aliquots with 50 mg/ml gentamicin (free base) are stored at -20 °C.
- Working solution: Dilute 1 ml of gentamicin stock in 1 l Barth's solution.
- Gentamicin sulfate salt, potency approx. 600 µg Gentamicin per mg.
- Alternatively to gentamicin, a mixture of penicillin and streptomycin can also be used to suppress growth of microorganisms.

### Barth's solution

- pH 7.4 (with NaOH)
- 88 mM NaCl
- 2.4 mM NaHCO<sub>3</sub>
- 1 mM KCl
- 0.33 mM Ca(NO<sub>3</sub>)<sub>2</sub> \* 4 H<sub>2</sub>O
- 0.41 mM CaCl<sub>2</sub> \* 2 H<sub>2</sub>O
- 0.82 mM MgSO<sub>4</sub> \* 7 H<sub>2</sub>O
- 5 mM Tris/HCl

Barth's solution without Ca<sup>2+</sup>

- pH 7.4 (with NaOH)
- 88 mM NaCl
- 2.4 mM NaHCO<sub>3</sub>
- 1 mM KCl
- 0.82 mM MgSO<sub>4</sub> \* 7 H<sub>2</sub>O
- 5 mM Tris/HCl

### Oocyte Removal

1. Remove the appropriate amount of ovarian tissue surgically from one side of the frog. Please refer to standard protocols on this subject (Stühmer and Parekh 1995).
2. Transfer the ovarian lobes into a new large Petri or cell culture dish (for example 100 mm Falcon) filled with Barth's without Ca<sup>2+</sup>.

## Collagenase Digestion and Defolliculation

Ovarian tissue contains immature and mature oocytes, as well as connective tissue from which the oocytes must be separated. Ovarian tissue should be removed completely by collagenase digestion. Oocytes are enveloped in a follicle cell layer which can cause trouble when plating oocytes into well plates. Remaining pieces of follicular tissue causes oocytes to stick to the walls. Oocytes will not move into correct positions in the middle of a well by themselves. Additionally, the follicular cell layer sometimes hampers the impalement by the injection needle causing damage to the oocytes or clogging of the tip. Therefore, it is important that isolated oocytes are completely free of the surrounding follicular cell layer. The whole procedure should be completed after about 2 to 2.5 hours. Please adjust the collagenase concentration if this is not the case.

1. Divide the tissue with a razor blade and a forceps into smaller, approximately 0.5 cm<sup>2</sup> large pieces.
2. Transfer the clumps into 50 ml Falcon tubes containing 40 ml of 1.5 to 2.0 mg/ml collagenase in Barth's without Ca<sub>2+</sub>. A volume of up to 7.5 ml of tissue can be put into a single tube. For more tissue, use additional tubes. Otherwise, the collagenase digestion would take too much time.
3. Place the tubes onto the shaker and let them shake gently at 20 °C. Check the progress after 90 min (and then every 15 min), and vigorously shake the tube briefly by hand to accelerate the process.
4. Typically, 120 minutes after beginning of the treatment, practically all oocytes should be isolated and the majority of them should have already lost their follicular cell layer. If not, continue the collagenase treatment for a maximum of 30 min.
5. Wash the oocytes extensively with Barth's solution (minimum of 5 times with 30 ml) to remove the collagenase completely. Then fill up the tube (approx. to 45 ml) and put it back onto the shaker for 10 minutes.
6. Change the solution to Barth's without Ca<sub>2+</sub> and put it onto the shaker again for another 10 minutes. Practically all oocytes should be defolliculated now. Shake the tube vigorously to remove the follicle cells completely, if necessary.
7. Wash the oocytes with Barth's solution until the supernatant is clear and free of follicular cell layer fragments.

## Selecting Good Oocytes

### Rough Selection by Filtration

1. Fill a 100 ml beaker with 80 ml of Barth's solution. Place the oocyte mesh sieve into the beaker for grading the oocytes by size. The mesh of the sieve should be completely immersed in the fluid.
2. Pipette an amount of oocytes onto the sieve. Approximately half the sieve should be covered with oocytes. Too many oocytes on the filter will lead to an inefficient filtration.
3. Gently move the filter about two centimeters up and down (in the fluid) to separate the oocytes by size.
4. Transfer the oocytes of appropriate size (that did not go through the sieve) into a 60 mm petri dish filled with Barth's + gentamicin.
5. The filtered oocytes are incubated at 18 °C for 1 h.

Note: The incubation step is necessary for identifying damaged oocytes in the next step.

### Fine Selection

Use a stereo microscope and the golf-club shaped glass tool to check each single oocyte for the following criteria.

Outer form:

No visible damage of the cell.

Well separated colors (dark and light brown).

No residues of follicular tissue.

Size:

Uniform size of about 1.2 mm.

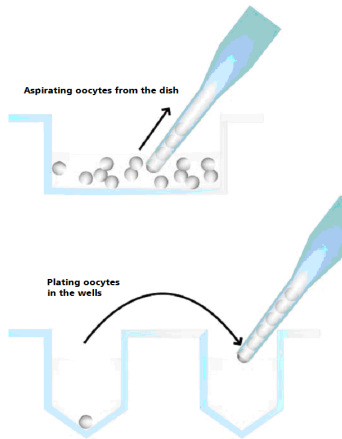
Note: Selecting oocytes is an important step. Perform it very carefully to obtain best results



## Plating Oocytes

You need a well plate filled with Barth's + gentamicin.

**Important:** Visually check all well plates before use. Use only unsterile and very flat plates. Sterilization may lead to warped plates. Warped plates will decrease the injection and expression efficiency.



1. Aspirate a number of oocytes with a transfer pipette by applying a low negative pressure, for example, with a rubber ball.
2. Move from well to well with the transfer pipette, carefully dropping one oocyte into each of the wells of the well plate. Grip only the glass pipette and do not squeeze the rubber ball, to avoid that too many oocytes will leave the pipette. The oocyte should drop into the fluid by its weight alone.
3. If occasionally more than one cell drops into a well, this can be corrected after the plating will have been finished.
4. Due to the higher density of the vegetal pole, the oocytes will usually settle with the animal pole facing up if the (sticky) follicle cells were completely removed. Immediately check the position of each oocyte under a stereo microscope, and correct it carefully with the golf-club shaped glass tool, if the orientation is not correct. A manual correction should be necessary for less than five percent of the oocytes if the follicle cells were completely removed.
5. Cover the well plate with its lid, and seal the rim with Parafilm to reduce evaporation, and store it at 18 °C until injection.
6. It is possible to inject the cells right after plating, but the oocytes should not be further disturbed, for example not be washed or otherwise treated until they have been allowed to adhere to the well bottoms (about 2-3 hours). The oocytes can also be injected on the next day, after being stored overnight at 18 °C. If injected on the next day, it is recommended to check the viability again under the microscope, and replace bad oocytes before the injection. Please note that oocytes sometimes express better when they are fresh and not stored overnight before injection.

# TECHNICAL SPECIFICATION

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## TECHNICAL SPECIFICATION

### General Information

Operating temperature	10 °C to 20 °C
Storage temperature	0 °C to 70 °C
Dimensions (W x D x H)	320 x 320 x 310 mm
Weight	23 kg
Power	30 W

### Power Supply

Power Supply Unit	FSP 150 ABA
AC Input	100 V to 240 V ~ 2 A @ 50 - 60 Hz
DC Output	24 V = 6.25 A

### Performance

Usable well plates	disposable standard 96 or 384 well plates, custom well plates on request
X / Y movement speed	80 mm/s
Z-axis speed	40 mm/s
Movement time from well to well	2 s
Average time needed for 96 injections	8 min
Positioning accuracy	20 µm in X / Y and Z dimension
Injection volume	1 to 100 nl in 0.5 nl steps

### Air Pressure

Supply pressure maximum	10 bar
Supply pressure working	3 bar

### Software

Roboinject software	Version 1.1.0
Connection to the computer	USB 2.0 High Speed
Operating system	Microsoft Windows® 10 or 8.1 with NTFS, English version

# CONTACT INFORMATION

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## CONTACT INFORMATION

### Local retailer

Please see the list of official MCS distributors on the [MCS web site](#).

### Mailing list

If you have subscribed to the [Newsletter](#), you will be automatically informed about new software releases, upcoming events, and other news on the product line. You can subscribe to the list on the contact form of the MCS web site.

[multichannelsystems.com](http://multichannelsystems.com)