




# *routine-realplex* Software

Software manual

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## 1 Introduction



This manual acts as a supplement to the instruction manual of the Mastercycler ep *realplex*. For a general understanding, please read the instruction manual of the Mastercycler ep *realplex* first. In this supplement, you will only find the features listed, which are characteristic for the routine-*realplex* software.

The so-called routine-*realplex* software represents a variant of the regular *realplex* software (version 2.0 and higher).

## 2 Overview

In the user administration the user group **Standard User** is replaced by the user group **Restricted User**.

Restricted users only have limited access to different software features. This restricted access and the consequences are described in this manual.

The user group **Administrator** did not noteworthy change.

### Restricted user:

- Restricted users have to work with predetermined templates under standardized conditions. After starting an assay, the assay name is assigned automatically according template name, date and time.
- Modifications of plate layout and PCR program are not possible. New assays can not be created on the basis of an empty plate layout. Templates and assays either have to be present in the database or can be imported into the actual database.
- Analysing of assays: Only the analysis modes **Quantification**, **Melting Curve** and **+/- Assay** with limited access to evaluation parameters are present. Endpoint measurements can not be performed. The feature **Edit Mode** is not accessible.
- Imported templates and assays are automatically stored in a predetermined folder. Simultaneously with exporting an assay, a report file is generated.
- Restricted users have no access to **Color Calibration** and **System Configuration**; only **Background Calibrations** are permitted.

### Administrator:

Changes concerning the administration level in the regular *realplex* software version 2.0 or higher include:

- Deletion of assays and templates of restricted users are allowed.

## 3 Installation

- ▶ For installation see the installation manual in the booklet of the software CD.

## 4 Operation

### 4.1 General operation as restricted user

#### 4.1.1 Login

After opening the program the **Login** dialogue appears.



After log in the restricted user has access to his folders, assays, and templates and can start his templates and assay setups on the connected Mastercycler ep *realplex*. Restricted users do not have the admission to view folders and assays of other users. Therefore, copying and analysing those data is not possible.

#### 4.1.2 User administration

Restricted users are not allowed to set up new users.

#### 4.1.3 System information

The dialogue **realplex System Info** can be viewed via the menu item **Setup**. Only an administrator has access to the **System Configuration** dialogue. Among other things the following details can be learned from the first dialogue:

- Users which had been set up by an administrator.
- Properties of cyler and cyler system as well as system info of *realplex* module, e.g. software versions, last user logged in.
- Logs and protocols like Cyler Protocol, ErrorLog, UserLog and *realplex* ModuleLog.

All these lists can be saved or printed.

#### 4.1.4 Calibration

Restricted users have only limited access to the calibration functions in the software.

**Color Calibration** is not possible and has to be performed by an administrator.

**Background Calibration** can only be made for these consumables/sealing combinations which are already present in the software and which had been calibrated by an administrator before.

- ▶ For background calibration select the menu item **Setup**.

The dialogue **Background Calibration** presents the actual background calibrations made on the instrument.

- ▶ In order to renew an existing background calibration, select the corresponding entry and press the function key **View/Run...**
- ▶ In the dialogue **Edit Background Calibration** there is only given the opportunity to start the calibration by the function key **Calibrate**. Follow the prompts and proceed as described in the instruction manual of the Mastercycler ep *realplex*.

### 4.1.5 Database tool

There are no restricted rights for the database tool. Configuration of the database tool is only accessible for an administrator.

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Assays and templates from *realplex* software versions 2.0 and higher can not be imported by software versions 1.5 or lower!

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For completion, the following features concerning operation of the Mastercycler ep *realplex* are not accessible to restricted users:

- Probe and dye administration.
- Show/Hide Navigator and Show/Hide Mini Plate Layout in the menu item **Setup**.
- Menu item **Edit** (therefore commands like **Copy/Save Results** can only be chosen via the right mouse button).

## 4.2 Assay setup as restricted user

### 4.2.1 Assay management

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A restricted user is able to start templates and assay setups which are stored in his own assay folders. Moreover, he can analyze completed assays.

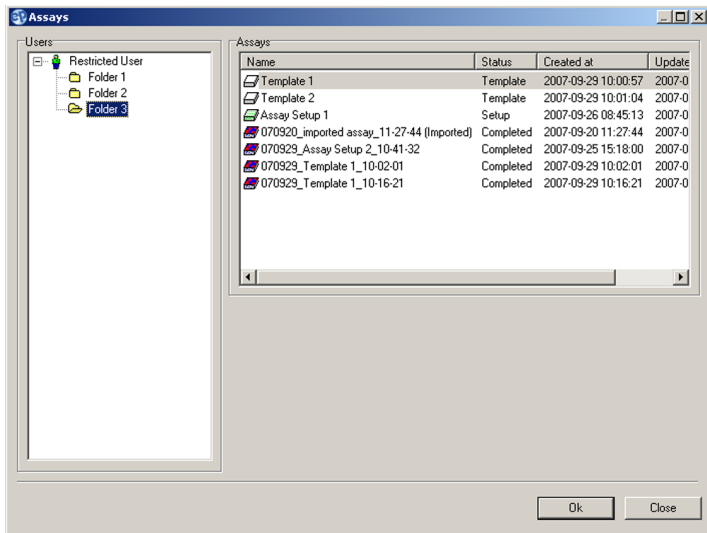
Templates and assays can both be imported and exported. Restricted users are not allowed to

- create new assays/templates,
  - make any changes to the plate layout and PCR program of templates/assays,
  - create own assay folders,
  - delete any assays, templates or folders,
  - copy assays/templates from other users.
- 

### Opening of templates/assays

1. By selecting the icon **Open Assay** in the tool bar or the menu item **File** the dialogue **Assays** is shown.

Templates, assay setups and assays are arranged in alphabetical order. Creating, deleting and renaming of assays and templates is not allowed.

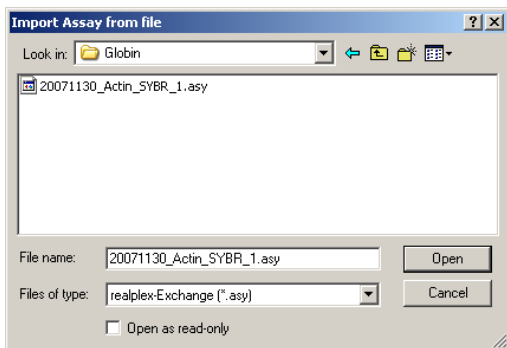


- Choose a template/assay and confirm the dialogue with **Ok**. Because no changes can be made by restricted users, all base settings of an assay, like dyes, sample volume, probe, background are already fixed and highlighted in grey. In addition, modifications of plate layout and PCR program are not possible. Only submission of a plate comment can be made. The comment is saved automatically.
- See separate description for analyzing completed assays (see *Analysis as restricted user* on page 11) and starting templates and assay setups (see *Starting templates* on page 10).

#### 4.2.2 Import and export of templates and assays

Import and export of template/assays are basic requirements in order to work as restricted user.

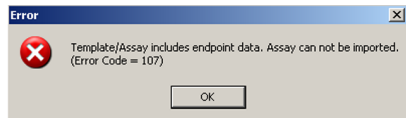
For new users the dialogue **Assays** includes no assays or templates. Because a restricted user is neither allowed to create new assays/templates nor to copy them from other users, he is prompted to import a template/assay by choosing the menu item **File**. Any template/assay can be imported by the dialogue **Import Assay from File**.



- ▶ After confirming the dialogue with **Open**, the template/assay is automatically saved in the corresponding folder of the restricted user.

This directory corresponds to the folder in which the administrator, who created the template/assay, saved it. Thus templates/assays are always irreversibly connected with a directory. If an appropriate folder is not present, it will be automatically generated. The restricted user does not have any influence on the protected location of templates and assays. If a template is imported, the original name will remain unaffected, whereas in the case of an assay, the appendix <imported> will be added. Overwriting of templates/assays will occur without prior notice, if templates/assays with identical names are imported.

Does a restricted user try to import an assay, which includes endpoint data the following error message occurs:

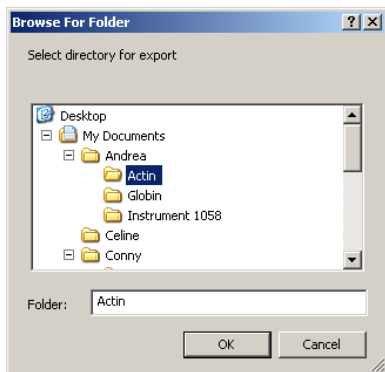


Import of template/assays is only possible, if the database in which the import should be carried out, possess the appropriate color calibration!

For exporting an assay/template, the data set has to be loaded to the analysis module.

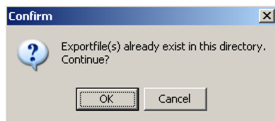
- ▶ By selecting **Export** under the menu item **File**, a directory can be chosen, to which the assay will be transcribed.





Assay names are not allowed to be changed.

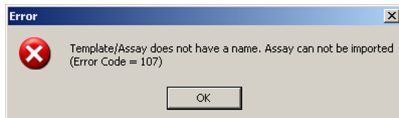
If there is already an export file present in the target directory, a software prompt will occur.



Together with the assay data a report is created in form of a PDF file (see *Report* on page 11). The PDF file generation for reports can be controlled by the administrator (see *General operation as administrator* on page 10).



Export of templates/assays to *realplex* software version 1.5 and lower is not supported!



### 4.2.3 Starting templates

- ▶ For starting a template or an assay setup, the file has to be loaded to the assay setup module (see *Opening of templates/assays* on page 6).

The sample tubes have been inserted into the thermo block, the heated lid is slid forwards. The sealing clamp is then moved down.

- ▶ The assay is started by selecting the icon **Start Current Cycler Program** from the tool bar (alternative: menu item **Command**).

In contrast to the regular *realplex* software, no background plate has to be chosen subsequently. The correct background plate has to be included in the template by the administrator, who has created the template. By starting a template, a corresponding assay name is automatically created by the software.

The following rules for automatically creating assay names are applied:

- The template name constitutes the inner part of the assay name.
- The template name is preceded with the actual date.
  - YYMMDD\_ (year/month/day)
- The template name is terminated with the actual time in the moment of starting the template.
  - \_hh-mm-ss (hour/minute/second)
- Example: 080113\_HCV1 fast cycling\_09-15-23

Assay names must not exceed 50 characters. If the template name is too long (>34 characters), the software reduces the number of characters in order that date and time can be added. Templates can be started as often as favored by the user.

All information for the ongoing PCR program is shown under **Monitoring** in the panel **Cycler Status**. Interruption, continuation and abort of the PCR program is permitted as for all other users.



Make sure that the instrument is warmed up for at least 15 minutes before starting an assay.

## 4.3 General operation as administrator

Operation as administrator is nearly identical to the behaviour in the regular *realplex* software. See two special features:

- User administration: In the routine-*realplex* software the administrator sets up restricted users instead of standard users. The level standard user is not provided. Several users with administration rights can exist.
- The administrator can delete assays and folders of restricted users.
- The administrator can prevent the automatic generation of reports by deactivating the checkbox **Automatically Generate PDF on Assay Export**. This checkbox is shown in the **Report Configuration** dialogue under **Edit**.

## 5 Analysis as restricted user



Restricted users have only access to the analysis modules quantification, melting curve analysis and +/- assay. The analysis modules relative quantification, endpoint and gene identification can only be used by a user with administration rights. While one assay is running, it is possible to analyze an assay that has already been completed under **Analysis**. Information about plate layout and PCR program can not be shown in this case. All given information in this chapter is specifically related to restricted users.

### 5.1 General

Assays which can be analyzed by a restricted user either were started on basis of a template or assay setup before or were imported by the restricted user (see *Import and export of templates and assays* on page 7).

After log in the restricted user is directly guided into the **Open Assay** dialogue. After selecting an assay and confirming the dialogue with **OK**, the data will be accessible under **Analysis** in the navigation tree. If the assay is finished shortly before and no other assay was opened afterwards, the data will be automatically accessible under **Analysis**. The following features in the analysis module are **not available** for restricted users:

- **Raw Data** view
- **All Dyes** view in the analysis mode in case of multiplex assays
- **Show All** and **Show Selected Samples** view in the sample table
- Display of legends
- **+/- Assays** with endpoint data
- **Relative Quantification**, **Gene Identification**, **Endpoint analysis**
- Report configuration

### 5.2 Report



In general, only data which have been analyzed before can be shown in the report.

A standard report which includes all relevant data of the assay (document information, plate layout, PCR program, charts and sample tables of Monitoring and Analysis) is generated automatically in parallel by exporting an assay (see *Import and export of templates and assays* on page 7). The resulting PDF file is given the name of the assay and therefore shows the same name as the exported assay file. Both, exported assay and report, are written to the same directory. Names of assays and reports can be changed in the Windows Explorer or an equivalent software tool. In consequence of the automatically generated report, access to report under the menu item **File** is cut off.

In comparison to a report generated by an administrator, there were made several minor modifications:

- Raw Data are not shown.
- Amplification plots in linear scale are not accessible.
- No option to see charts with all used dyes.
- If an assay contains one or several subassays, all these subassays will be presented in one report. If a subassay has not been analysed before, default analysis setting will be used.

The PDF file generation for reports can be controlled by the administrator (see *General operation as administrator* on page 10).

### 5.3 Save analyses

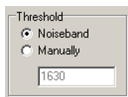
Saving of all changes made during analyzing an assay are done automatically and without any direct intervention of the restricted user.

### 5.4 Quantification

After completing an assay or opening a completed assay the quantification data are loaded to the work area under **Analysis** (combo box **Type of Application: Quantification**). By default the fluorescence curves are shown in logarithmic scale. For linear display, the check box **Log Scale** must be unchecked. An internal reference dye can not be taken into account. The Mastercycler ep *realplex* does not need an internal reference dye, like ROX, to give accurate results.

#### 5.4.1 Determination of threshold

The threshold is used to specify  $C_T$  values of samples. The  $C_T$  value is the cycle in which the fluorescence signal intersects with the threshold. Whereas the administrator has access to four different methods to generate  $C_T$  values, restricted users can only analyze quantification data by the threshold settings **Noiseband** and **Manually**.

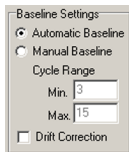


If a restricted user imports an assay which was analyzed by the setting **CalQplex** or **Best R<sup>2</sup>**, the assay will be reanalyzed and presented as **Noiseband**.

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### 5.4.2 Determination of baseline

The settings for baseline and drift correction are the same as known from the regular *realplex* software.



All settings made in the panel **Baseline Settings** affect the curve shapes and the  $C_T$  values.

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The following features of the software are handled just like in the regular *realplex* software:

- Standard curves
- Results shown in sample tables
- Multiplex analysis

## 5.5 Melting curve analysis

Select **Melting Curve** in the combo box **Type of Application**. All settings are the same as in the regular *realplex* software.

## 5.6 +/- Assay

Data of a PCR can be analysed in the analysis module **+/- Assay**. All settings are the same as in the regular *realplex* software. The analysis of endpoint data is not possible.



For information concerning maintenance and technical data of the instrument as well as general troubleshooting please read the instruction manual of the Mastercycler ep *realplex*.

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## 6 Ordering information

Order No. (International)	Order No. (North America)	Description
6302 900.904	on request	<b>routine <i>realplex</i> software</b>

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