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Installation
Installation

How to install MicroBeta² Workstation software from CD

MicroBeta² Workstation software is pre-installed on your MicroBeta² instrument’s built-in computer, so you do not need to perform any installation in order to get started. If you would like to install the software onto a separate computer, for example, for familiarization or training purposes, or if you should ever need to re-install the software onto the instrument’s built-in computer, installation is straightforward.

1. Insert the installation CD into the appropriate drive. The installation program will start automatically (if this does not happen, run Setup.exe from the CD). The dialog shown below will appear.

![MicroBeta Software Setup](image-url)
2. Start by clicking the first of the listed installation components, **.NET Framework 3.5**

As the installation proceeds you will see the status of events, and will be invited to confirm certain processes.

3. On successful installation of **.NET Framework 3.5**, click the next component, **SQL Server 2005 Express**

4. After this, install **GSC Components**

5. Finally, install **MicroBeta Software**

If you have purchased the 2450-3010 Enhanced Security mode option (see page 13) and have the product key, you can install it during the MicroBeta software installation.

6. Click the Enhanced Security option check box and provide the product key
The software will guide you through the installation of the Enhanced Security option.

If instead you want to install in normal mode, leave the check box unselected

**How to add users to a User Group (Enhanced Security mode only)**

In order to use the MicroBeta² Workstation software, all users who are going to login to Windows must be added to the Windows user group, MicroBeta2Users.

1. To do this, login in as Administrator and open Control panel/Administrative button s/Computer management.
2. Select the existing MicroBeta2Users - user group by double clicking it.

The MB WIW Users Properties dialog will be
displayed.
3. Click the **Add** button to add users to the group. The **Select Users** dialog will be displayed.

![Select Users Dialog](image)

4. Check that **From this location** is your computer name and not e.g. the domain of the computer. Use the **Locations** button to choose your computer if needed.

![Select Users Dialog](image)
5. Click the **Advanced** button. Another **Select Users** dialog is displayed.

6. Click the **Find Now** button. The list in the lower part (see next picture) is populated with local users.

![Select Users dialog](image)

7. Select the users from the list who will use the MicroBeta² WIW application.

You can select several users by keeping the Ctrl-key down while clicking the user names.

8. Click **OK**.
You can repeat the above steps to add more users in the list.

9. When the list is ready, click **OK** then **Close** the remaining windows.

*Note!* The abbreviation WIW, used occasionally in this manual, stands for Windows Workstation.

*Note!* In normal use it is expected that the MicroBeta² Windows Workstation software and other related tools will be the only applications run on the system computer. PerkinElmer offers no guarantee whatsoever as to compatibility of the MicroBeta² Windows Workstation software and associated tools with other software, including updates to the operating system, installed or run by the user on the system computer.

*Note!* Should the system be connected to the internet, the administrator will be responsible for ensuring that protection by a proper firewall and appropriate antivirus software is available. By default, the Microsoft Windows firewall is turned on.
Introduction
Introduction

Overview

MicroBeta² is a multi-detector plate counter for liquid scintillation and luminescence measurements. It can measure samples on microplates (both flexible and rigid), filtermats and tubes. It also gives excellent performance for Scintillation Proximity Assays. Plates can be loaded in a stacker or a robot system can be used. As a luminometer with injectors (MicroBeta² LumiJET) it can measure both flash type and glow type luminescence.

To be able to use injectors, you must have an "injector ready" MicroBeta². However, an injector-ready instrument may still not have an injector module installed. See the instrument manual for information about installing an injector module.

Enhanced Security mode and 21 CFR Part 11

21 CFR Part 11

The regulation (21 CFR Part 11) sets the criteria under which the Food and Drug Administration (FDA) of the USA considers Electronic Records and Electronic Signatures (if applied) to be trustworthy, reliable and generally equivalent to traditional paper records and handwritten signatures executed on paper. The regulation applies to records in electronic form that are created,
modified, maintained, archived, retrieved, or transmitted, under any records requirements set forth in agency regulations. It also applies to electronic records submitted to FDA under requirements of the Federal Food, Drug, and Cosmetic Act.

**Enhanced Security mode**

MicroBeta$^2$ has an Enhanced Security mode intended for facilities that have to comply with the 21 CFR Part 11 regulation from the FDA. During installation you can select whether you want to use the Enhanced Security mode. Once you have enabled this mode you cannot disable it because to do so would not be in compliance with 21 CFR Part 11.

The Enhanced Security mode for MicroBeta$^2$ provides the technological controls and features to support full 21 CFR Part 11 compliance. These features can be classified under three main headings:

- **Improved access control (unique UserID/Password combination) with five user levels**
- **Improved file security covering protocols (versioning) and result files**
- **Audit trails of user actions**

This manual and the electronic help will tell you how to use MicroBeta$^2$ with or without the Enhanced Security mode. All differences in use are noted and explained.
**Warning!** Use of the Enhanced Security mode does not alone ensure compliance with 21 CFR Part 11. Any facility that wants to be compliant with the 21 CFR Part 11 regulation must also implement the necessary procedures and controls set by the regulation.

**User groups in Enhanced Security mode**

There are five user groups with different user rights. A user can belong to several user groups. The default user groups and default rights of each group are as follows:

<table>
<thead>
<tr>
<th>User group</th>
<th>Rights</th>
</tr>
</thead>
<tbody>
<tr>
<td>WIW administrator</td>
<td>Add and remove users and set the groups</td>
</tr>
<tr>
<td>WIW system manager</td>
<td>Permanently delete files, edit system settings, and use terminal window</td>
</tr>
<tr>
<td>Auditor</td>
<td>View the audit trail</td>
</tr>
<tr>
<td>Editor</td>
<td>Create, modify and delete protocol files and delete result files and protocols</td>
</tr>
<tr>
<td>User</td>
<td>Run protocols (counting, normalization, standardization)</td>
</tr>
</tbody>
</table>

To understand how to assign users to groups, please refer back to the Installation section and page 5.
Order of Operation

Order of operation of MicroBeta$^2$ can be divided into a number of main phases, which are briefly introduced in the following paragraphs and described more fully at the page references given. The main phases are preparation and corrections (see pages 27 and 55), which only need to be performed once, operation (see page 105) and results management (see page 99). There are also a number of miscellaneous functions that help you set up MicroBeta$^2$ in the way you want for e.g. communicating with an external computer, backing up result files etc.; these are described in System operations (on page 107).

Preparation phase

Injector setup
This operation is described on page 27.

*Note!* This is necessary only if you are using the injector system. The injector(s) must have first been installed.

Assay protocol setting
Counting of samples is controlled by a set of parameters called an assay protocol. Protocol setting is the process of defining the parameters in a protocol. An assay protocol will also include detector normalization or quench correction information with or without crosstalk correction, see below.
Corrections phase

Detector normalization is necessary when multi-detector MicroBeta\textsuperscript{2} instruments are used for CPM or luminescence counting. It is the procedure by which the slight variation between detectors is calculated and correction factors are produced. These factors are used by MicroBeta\textsuperscript{2} to ensure the results obtained from any of the detectors are equivalent.

Quench correction is an extended form of detector normalization and is needed for DPM counting where, in addition to the detector normalization, it is necessary to correct for the quenching of samples.

The needed detector normalization or quench correction information is obtained by preparing and running special protocols. These are described in the section starting on page 55.

In the case of microtitration plate cassettes that do not provide optical shielding between wells, it is necessary to correct for the light coming from adjacent wells and contributing to the counts detected from any particular well. If selected, crosstalk correction is included in the detector normalization and quench correction procedures (see page 68).

Luminescence counting requires a special detector normalization procedure (see page 70).

When you have finished setting protocols for the types of samples and counting you need, and have performed the
necessary detector normalizations or quench corrections, you are ready to operate MicroBeta².

**Operation phase**

MicroBeta² is very easy to operate: you just fit your plate into a suitable cassette and fix a barcode on it. The barcode will tell MicroBeta² which protocol to use in counting the plate. You then load the plate(s) into the cassette rack and click the **Start** button on the screen of your PC. MicroBeta² will count the plate and produce the results according to the instructions you have given in the assay protocol or the normalization or standardization parameters. See page 105 for full information on operation.

**Results management phase**

Results are produced as printout and/or files that can be viewed or imported into other programs. Result handling is described in detail starting on page 99.

**How to start the counter**

1. Switch on the printer

2. Switch on MicroBeta² with the power switch at the back of the counter.

3. Start up the MicroBeta² Windows Workstation program by clicking the icon in the Program manager
When the Windows workstation software is started up a Login dialog is displayed. This requests a username and password.

4. Type in your user name and password

5. Click OK

The software checks the validity of the username and password. After a successful login the software can be used according to permissions associated with your user name user. Clicking the Cancel button closes the software if no protocols are running.

**Brief guide to the user interface**

The MicroBeta² Windows workstation software is a 32 bit application running under Windows XP.

The main window consists of five main parts: the **title bar** [A], **menu bar** [B], **toolbar** [C], **client area** [D] and **status bar** [E]. The client area may be completely filled with any of the child windows.
Title bar
The main title bar has the program title in it and it allows you to drag the window around on the screen. Double clicking the title bar toggles the main window between maximized and restored size. When a child window is maximized, the title bar shows also the title of the child window.

Menu
The menu allows you to give commands to the program by keyboard or mouse. The menu titles are: File, Counter, View, Window and Help. You seldom need the menu if you use the mouse since the main commands are on the toolbar.
Menu items that may be unavailable
The following table shows the restrictions in Enhanced Security mode

**File menu items**

<table>
<thead>
<tr>
<th>Item</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injector Setup</td>
<td>Enabled only for WIW User group members</td>
</tr>
<tr>
<td>Communications, Sharing and Service</td>
<td>Enabled only for WIW System Manager group members</td>
</tr>
<tr>
<td>Reset Run ID</td>
<td>Disabled for all users</td>
</tr>
</tbody>
</table>

**Counter menu items**

<table>
<thead>
<tr>
<th>Item</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start, Stop, Pause, Next Position</td>
<td>Enabled only for WIW User group members</td>
</tr>
</tbody>
</table>

**Toolbar**

The toolbar contains buttons for commands that are used frequently. The same commands are also in the menu. If a button is not operable, the text of the button is gray. The traffic light on the Start button shows green when the counter is in the Ready state and red when it is not. The traffic light shows red when: the counter is counting, the program is setting counter parameters or the counter is off. In addition, if you have temporarily halted counting by pressing the Pause button, the Start button changes its name to Continue, and the traffic light shows yellow. You can hide or show the toolbar by selecting Toolbar from the View menu. Hiding the toolbar gives more room for your windows. The toolbar is not required for running the
program. You can give the same commands from the menu.

The following shows a list of button operations:

<table>
<thead>
<tr>
<th>Button</th>
<th>Menu command</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start*</td>
<td>Counter-Start</td>
<td>Starts counting in automatic mode</td>
</tr>
<tr>
<td>Stop*</td>
<td>Counter-Stop</td>
<td>Stops counting</td>
</tr>
<tr>
<td>Pause*</td>
<td>Counter-Pause</td>
<td>Pauses counting</td>
</tr>
<tr>
<td>Live</td>
<td>View-Live display</td>
<td>Brings live display (or Window-Live display) to the top</td>
</tr>
<tr>
<td>Protocols</td>
<td>File-Protocols</td>
<td>Shows the list of protocol groups for selecting and opening</td>
</tr>
<tr>
<td>Results</td>
<td>File-Results</td>
<td>Shows a dialog containing the latest results. Max. of 100 results shown</td>
</tr>
<tr>
<td>JET*</td>
<td>File-Injector setup</td>
<td>Gives access to the injector system functions</td>
</tr>
<tr>
<td>System</td>
<td>File-System</td>
<td>Leads to system settings</td>
</tr>
<tr>
<td>Print</td>
<td>File-Print</td>
<td>Prints the active document</td>
</tr>
<tr>
<td>Help</td>
<td>Help-Contents</td>
<td>Displays help</td>
</tr>
</tbody>
</table>

* See "Menu items that may be unavailable" (above)

**Status bar**

The status bar at the bottom of the main window shows the status of the counter. If the counter is in the Ready state, it reads READY. If the program is waiting for the counter to go to the Ready state, the status bar reads WAITING READY. During counting it shows a variety of status information sent by the counter. You can hide or show the status bar.
selecting Status bar from the View menu. Hiding the status bar gives more room for your windows. The Status bar is not required for running the program, but it may be handy because it lets you see the state of the counter when counting.

The left area of the status bar describes the actions of menu items as you use the arrow keys to navigate through menus. This area similarly shows messages that describe the actions of Toolbar buttons as you depress them, before releasing them. If after viewing the description of the Toolbar button command you wish not to execute the command, then release the mouse button while the pointer is off the Toolbar button.

**How to work without a mouse**

If you wish to work without a mouse, you can access any available menu item from the keyboard.

1. First press the **Alt** key
   This will highlight the leftmost menu title.

2. Use the cursor keys to move the highlighting to the menu title you want, then press **Enter**
   A menu will "drop down".

3. Either use the cursor keys to move down to the menu item you want and then press **Enter**, or press the letter on the keyboard corresponding to the underlined letter in the menu item you want to select.
Within a dialog you can use the **Tab** key to move from field to field. **Shift Tab** takes you in the opposite direction. The **Enter** key allows you to give a positive response to the dialog and the **Esc** key to cancel it.

**How to change the formats of date and time**

The program uses the format settings of the Windows XP operating system. If required you can change these settings.

1. Open Control Panel and double-click the Regional and Language Options icon.

2. Make your desired settings and click **OK**

3. Restart the workstation program to make the changes visible.
Preparation
Preparation

Injector preparation

This section is only applicable if you have the injector system installed. If you do not, then you should skip forward and continue with Protocol groups on page 30.

How to set up the injector(s)

The MicroBeta² LumiJET injectors need setting up before they can be used.

1. Click the **Injector** button or select **Injector Setup** from the File menu.

*Note!* Both the button and menu item will be disabled if the counter does not support LumiJET features or if the counter is not Ready.

The Injector Setup dialog has the following fields in it for setting parameters:

- **Injector assembly group**: For moving the injector assembly in and out.
- **Injector module group**: The buttons here control operation of the injector
- **Preparation / Maintenance**: These sets of buttons are for preparative operations and those that are performed after use of the injector
The software checks if the injector assembly is in or out and enables the **Injector Assembly** buttons accordingly.

2. Make sure the injector assembly is moved away from the counting chamber, either into the injector chamber on the left of the instrument, or removed from MicroBeta. Click **Move Out** if that button is active.

*Note!* In robot loading MicroBeta² LumiJET systems, the injector assembly must be moved to the measuring chamber before counting starts. So then the **Move In** command must be clicked.

3. Make **Preparation** settings as required.
More information on them is to be found in the Help.

4. Click Close

**How to look after the injectors**

After use, it is important to perform the following steps.

1. Save unused reagent by clicking the **Recover** button
2. Wash tubing with 50% ethanol solution and rinse with distilled water

*Note!* The exact procedure followed might depend on the reagent used. An alternative practice should be followed if recommended by the reagent manufacturer.

3. As the final step, empty the tubing
4. Keep the dispensing head dry and the fibre optic clear. Clean with a moist cloth, and always use distilled water.

**How to guard against air bubbles in tubing**

Air bubbles in tubing cause droplet formation on top of the microplate and add variation to assays. In dispensing applications it is thus essential to ensure that air bubbles are not present in tubing. This can be achieved by gas stabilizing the solutions at a higher temperature prior to starting assays, or vacuum treating the solutions, or by bubbling helium through the solutions. With viable cell applications the cell media should be gas stabilized for at least 3 hours in +37°C with the vial open. When stabilized back to room temperature and used during the same day, the solution does not create bubbles in the tubing.
Protocol preparation

How to open a protocol group

Every assay protocol belongs to a single protocol group. There can be many protocol groups, each representing a different application or different user. Counting protocols, which are the main topic of this section, are found in the General protocol group. A counting protocol contains information in it, which links it to a detector normalization or quench correction protocol. To help your work a selection of pre-installed protocols is supplied. These are listed in Appendix B, page 127.

1. To open a protocol group, click the Protocols button or select Protocols from the File menu.

The protocol group window will open showing the available groups. If additional groups are required they may be created by clicking System (see page 105) and choosing System view and Protocol Group Templates.
In addition to the **General** protocol group there are special **Detector normalizations** and **Quench corrections** groups. The protocols belonging to these groups have preset plate maps, which show how to place standards. For more details see the section on Detector normalization and Quench correction starting on page 55.

There is also a special group that may appear called IPA. This allows you to check the instrument performance. See the chapter on IPA, starting on page 113 for more details.

2. Select the group you want from the list and click **Open**.

A protocol group window, as described in the section on the following page, will open.

*Note!* The **Deleted protocols** group appears only in 21 CFR Part11 mode. If you select it, the Deleted protocols dialog appears (and not a protocol group window).

This shows the protocols that have been deleted from the Protocol group window. You can view the **History** of the protocol and **Restore** the deleted protocol. See History on page 45 for more details.
Protocol group window

The selected protocol name appears in the top left field, and to its right you see its protocol number. There can be up to 100 protocols in any protocol group, and they are automatically numbered as you create them.

The protocol name is preceded by one of the following code marks:

_ No detector normalization or quench correction data exists for the protocol (or the detector normalization or quench correction protocol assigned has not yet been run to get the data)

n A detector normalization or quench correction has been run
A detector normalization or quench correction with crosstalk correction has been run.

The pane at the bottom left hand corner of the window lists the parameters of the currently selected protocol showing at a glance what type of protocol it is. You can use the scroll bar to display the remaining parameters.

There are four main functions that are available through the Protocol group window:

**Protocol editing**
There are three buttons: Protocol, Plate map and Output. Protocol editing forms the next chapter in this manual, see page 35.

**Protocol management**
There are four buttons: New, Copy, Delete and History. Protocol management is described later in this manual, starting on page 43.

**Results management**
There are three buttons: Open, Copy and Delete. Results management operations are described beginning from page 100.

**Manual start**
There is one button: Start. Manual start is described on page 90.

*Note!* In Enhanced Security mode the Protocol group window has the following restrictions:
The **Start**, **New**, **Copy** (protocol), **Delete** (protocol and results) and **Template** buttons are enabled only for users belonging to the WIW Editor group.

The **Copy** (results) button is enabled for users who belong to the WIW User or WIW Editor groups providing that no protocols are currently being run.

*Note!* If all the buttons except Protocol are disabled it means that this group has been defined as a system group (e.g. an IPA group) in System view.

**How to print protocols**

A number of protocol printing options are available.

1. When the protocol group window is open, click the **Print** button

   ![Print Selection Dialog]

   A contents selection dialog is shown with three options.

2. Choose your preferred option and click the **OK** button
Note! The status character ('_', 'n' or 'c') of a detector normalization or quench correction is printed with the title "Status" when the protocol listing and list of protocols is printed.

The fonts for printout can be selected from System view, see System operations on page 105.

Protocol editing

In the protocol group window there are three buttons in the Edit group to enable you to edit the settings of individual protocols. Protocol takes you to the counter parameters editor where, amongst other things, you will be able to specify which detector normalization or quench correction protocol your counting protocol will use. Plate map takes you to the plate layout editor (see page 42), and Output allows you to make the output settings (see page 47).

How to set protocol parameters

To start the Counter parameters editor proceed as follows.

1. With a protocol group window open, click the Protocol button

This editor consists of five tabs, General, Corrections, Counting Control, JET (if this is installed) and Other.

2. Make your desired parameter settings on the various tab pages shown on the following two pages

The different parameters are described in the Help.
Preparation
If you are working in Enhanced Security mode, the **Add comment** dialog pops up every time you make changes to the protocol.

3. Type a comment (reason) for the current version or leave the Comment field empty.

4. Click the **OK** button to save the comment and close the dialog.

5. After editing is complete, click **OK** on the last of the tab pages to accept editing or **Cancel** to discard all editing.

*Note!* In Enhanced Security mode the **OK** button is only enabled for users who belong to the WIW Editor group.

If **OK** is clicked, the assay protocol is saved on the disk. If the counter is in the Ready state, the assay protocol parameters are sent to the counter immediately, otherwise the parameters are sent when the counter next enters the Ready state.
**Luminescence protocols**

Editing luminescence protocols and liquid scintillation protocols are similar in most respects.

In a luminescence protocol **Lumin** will be selected as the label.

1. Select an appropriate luminescence detector normalization protocol

2. Under the **Corrections** tab and **Background correction** select **From norm**
If you have injectors installed and wish to work with flash-type luminescence reagents, you need to check the JET tab.

3. Check that **Use injector module(s)** is selected

4. Edit the other parameters as required

The **Keep cells in suspension** option makes it possible to use the dispenser to mix the suspension cell reservoir. When activated, the pump module 1 is used at frequent intervals to aspirate cell solution into the syringes and push it back to the reservoir. The resulting liquid flow
mixes the cell suspension and prevents cells from settling to the bottom of the vial or aspiration tubing. The mixing step is performed once before the assay, every time after a dispensing step and once every minute when the assay is in pause mode or waiting for a new plate. This mode requires a minimum volume of cell suspension solution, which is 500 µL times the number of syringes in the module 1. In a 12-detector unit this is 12 x 500 µL = 6 mL plus some extra to prevent aspiration of air.

When two dispensing steps are selected the Aequorin dual screen mode is enabled. This is especially helpful in Aequorin applications where antagonists are also screened. In this mode the plate is measured in two sequential steps. First cell suspension is dispensed to the wells and potential agonist signal is measured. This is performed for all selected wells in the plate. As a second step the agonist solution is dispensed in the wells followed by a measurement for potential reduction in the control signal caused by a possible antagonist effect of the compound. The time between these two dispensing steps depends on the measurement time, plate format, amount of measured wells and number of detectors. With a 12 detector instrument and a full 384-well plate the time between cell dispense and second agonist dispensing step is about 15 minutes with a 25 second total measurement time per well.
Plate map editing

The second button in the Edit box of the Protocol group window is **Plate map**. The Plate map shows the sample coding and allows you to edit it. Plates are shown graphically. Samples can be dropped into and removed from plates as you wish.

*Note!* In the Enhanced Security mode the **OK** button is only enabled for members of the WIW Editor group.

Plate map allows you to design (or use predefined) sample types that match as close as possible to your actual samples. You can put replicates of the samples into any position on any plate. The program does not impose any restriction on replicate position or order (if results are output to another program, some restrictions may apply).
Sample coding can be passed on to other programs. The positions of the samples are also sent to the counter, so it counts only the positions that are used, cutting down the measuring time. See the Help for a detailed description of the plate map features.

**Protocol management**

In the Manage group there are four buttons for managing assay protocols, **New**, **Copy**, **Delete** and **History**.

![Protocol management interface](image)

**How to create a new protocol**

If you want to create a completely new assay protocol, you need a template

1. Click the **New** button

The template that opens will contain certain default values.
2. When you have set the values you want, provide a name for the protocol and save it by clicking **OK**.

**How to copy a protocol**

You may wish to make a copy of an existing protocol, for example, if your existing protocol provides a better template for your purposes for editing.

1. Select the protocol you want to copy from the list

2. Click the **Copy** button

It will be given the name Copy of xxx where xxx is the name of the protocol copied. A dialog similar to the counter parameters dialog appears to allow you to edit parameters.

3. When you have made the changes you want, save this protocol under its new name by clicking **OK**.

**How to delete a protocol**

To delete a protocol, proceed as follows.

1. Select the protocol to be deleted from the list

2. Click the **Delete** button

3. Click **OK** to confirm the delete

The protocol will be removed from the list.
History

The Protocol history browser shows the history of the selected protocol. You can view the Details of the protocol or Restore the protocol. The Current version is also shown but you cannot restore it. The Restore button is enabled only for the person who has the rights to edit WIW. After you restore and close the Protocol history browser you can use the restored protocol or edit it in the Protocol group dialog.

Details
The Protocol Version Details dialog shows the protocol parameters for the current version.
Preparation

**Restore**
You can use the **Restore** function to produce the selected protocol version i.e. a new version of the protocol is created.

The protocol version is added to the protocol group of the current protocol, regardless of the protocol group to which it originally belonged.
Output definition

The third button in the Edit box of the Protocol group window is Output. Clicking it gives the Output dialog.

Under the General tab of the Output dialog there are six check boxes. Each of these corresponds to a tab page that allows you to edit the relevant parameters. The parameter fields are only enabled when the appropriate check box is
selected on the **General** page.

In the Enhanced Security mode the **OK** button is only enabled for users who belong to the WIW Editor group.

The **General** page shows which functions in the protocol output are enabled and which are disabled. There is also a short summary of the selections.

When printing is selected by checking the **Print** checkbox the printer is identified and the print items page is summarized. You are also told whether the printing is done online or after the whole assay is completed, whether the format is in a column and/or as a plate. The selected layout is also specified, e.g. the results of two plates can be printed on one A4-page in plate-format.

In the case of filing, two different files with each with its own file format can be produced at the same time e.g. **File1** in ASCII format and **File2** in Excel format.

When filing is selected, the following information is shown: the file type, the file items summary, the format (column and/or plate) and the full path and file name for the produced results.

For the result post processing requirements are selections: There is also a checkbox **Execute after File1 (or File2) output**. This allows selections affecting what is to be done with the results i.e. you can select an application
program to start automatically after the result file is completed.

In a case such as when e.g. the local disk drive is full and filing cannot be done an error is detected. The **Execute on error** checkbox allows an application program to be started automatically, which e.g. sends a network message to the user.

**Naming the output files**

You can decide on the format of output files, by specifying which fields the names should be built from.

1. Either select a format from the drop-down menu or type in a new format according to the rules below
The fields have the syntax `<name:width>` where the name is the name of the field and the width is the output width specification of the field. The name must be written in lower case characters. You do not need to specify a width, but it is advisable to do so if a certain format is required for subsequent data processing. In the enhanced security mode the default width is 7. In the Normal mode the default width is 3.

You should also remember that the actual characters from the field that are used in the name will depend on whether the field is for string or numerical type data.

The string data fields are: `protname`, `owner`, `counter`, `idshort` and `idlong`.

Let us say, for example, that `<protname>=Test
Then `<protname:2>=Te (with just the first 2 characters taken), while `<protname:6>=Test__ (with two dashes added at the end to make up the specified length).

The numerical data fields are: `runid`, `protid` and `plateindex`.

If, for example, `<runid>=1234
Then `<runid:2>=34 (with the 2 last figures taken), while `<runid:6>=001234 (with zeros added before the number).

The following table lists the fields available. You may combine two or more of these in whatever order you please.
<table>
<thead>
<tr>
<th>Field name</th>
<th>Replaced with</th>
</tr>
</thead>
<tbody>
<tr>
<td>proname</td>
<td>Name of the protocol</td>
</tr>
<tr>
<td>protid</td>
<td>ID of the protocol. Padded left with zeros</td>
</tr>
<tr>
<td>owner</td>
<td>Owner of the protocol from protocol parameters</td>
</tr>
<tr>
<td>runid</td>
<td>The sequence number of the run of the protocol. Width defines how many digits are used. Padded left with zeros</td>
</tr>
<tr>
<td>counter</td>
<td>Counter name from System-Settings-Counter</td>
</tr>
<tr>
<td>errmsg</td>
<td>Error message. Valid only in Execute when error settings</td>
</tr>
<tr>
<td>plateindex</td>
<td>Index of the plate in assay. First plate is 1. Valid only in file output settings for File 1 and File 2. Padded left with zeros</td>
</tr>
<tr>
<td>idshort</td>
<td>Plate ID if the plate ID reader is used and barcode is on the short side of the plate. Valid only in file output settings for File 1 and File 2</td>
</tr>
<tr>
<td>idlong</td>
<td>Plate ID if the plate ID reader is used and barcode is on the long side of the plate. Valid only in file output settings for File 1 and File 2</td>
</tr>
</tbody>
</table>

This and all of the output dialog features are described in greater detail in the Help.
Corrections
Corrections

Detector normalization

Multi-detector versions of MicroBeta\textsuperscript{2} have 2, 6 or 12 detectors, which make it possible to count the respective number of samples simultaneously. In order for the results from each detector to be equivalent, irrespective of small variations in efficiency and background between detectors, it is necessary to determine the relative efficiency and background of each detector and then correct for it. This is called detector normalization.

Note! Detector normalization may be performed even on single-detector counters, since the detector normalization protocol also allows you to perform crosstalk correction or background correction or calculate absolute counting efficiency.

Detector normalization is done by firstly measuring an optional background plate followed by one or two standard samples with defined activity or CPM in each detector.

Once CPMs have been measured, the relative efficiencies can be calculated. The efficiency of the detector giving the highest count rate is then defined to be one and the other detector efficiencies are expressed as a fraction of this value. These fractions are called efficiency coefficients.

Note! We are here discussing the measurement of radionuclides. For luminescence labels detector
normalization is also performed, and in this the efficiency average is set to 1.0. For more on Luminescence normalization see page 70.

When measuring a sample with a particular detector, the CPM of the sample is corrected by dividing the CPM by the efficiency coefficient. In dual label counting, corrections are made using dual label correction formulae (see Calculation methods in the Instrument manual).

When detector normalization is done the results are stored with the normalization protocol. The normalization data can be used by one or several protocols and are selected when editing the protocol.

**When is detector normalization necessary?**
Each counting or assay protocol requires detector normalization data to be stored before it can calculate corrected CPMs for the samples counted. A fresh detector normalization must be done when a new nuclide is counted or when counting features, such as nuclide or window, are changed. When a new type of microtitration plate is used, detector normalization with crosstalk correction may be necessary (see page 68).

**Preparing detector normalization samples**
A MicroBeta² instrument is supplied from the factory with a background plate and a detector normalization sample plate containing standards for $^3$H and $^{14}$C. The background plate is optional and only measured if selected in the protocol setting.
On the factory-produced normalization plate, the activities of the standards are supplied, but you do not need to use these given activity values unless you wish to calculate absolute detector efficiencies (for DPM results or for IPA purposes).

When making your own detector normalization plates, take care that the samples are in the correct positions so that they can be measured by every detector. The positions cannot be changed by modifying protocol parameters.

In 24-well plate single label counting the general rule is that the standard should be in position D5. As an exception to this, when the measured nuclide is $^{14}$C, the standard must be in position D6. This is because the factory-made detector normalization plate is intended for both single label $^3$H and $^{14}$C normalizations and dual label $^3$H/$^{14}$C normalizations. Hence the $^{14}$C standard always takes the nuclide 2 position.

For best results if 96-well sample plates are to be counted, and always for 12-detector counters, 96-well plates should be used as detector normalization plates.

For a 1-6 detector counter the standard for nuclide 1 must be in G11 and for nuclide 2 in H12. For a 12-detector counter and single label detector normalization the standard must be in G11, but for dual normalization the nuclide 1 standard must be in G10 and the nuclide 2 standard in G12. See the table below.
Sample positions on a detector normalization plate

<table>
<thead>
<tr>
<th></th>
<th>24-well</th>
<th>96-well</th>
<th>96-well</th>
<th>384-well</th>
<th>384-well</th>
</tr>
</thead>
<tbody>
<tr>
<td>(12 Det.)</td>
<td>(12 Det.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Single</td>
<td>Dual</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nuclide 1 standard</td>
<td>D5</td>
<td>G11</td>
<td>G11</td>
<td>G10</td>
<td>N22</td>
</tr>
<tr>
<td>Nuclide 2 standard</td>
<td>D6</td>
<td>H12</td>
<td>G12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>¹⁴C single label</td>
<td>D6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

See the section beginning on page 68 for crosstalk sample positions, and the section beginning on page 70 for luminescence sample positions.

**Quench correction**

Several samples labeled with the same activity can give different count results even in the same detector because of the difference in the amount of energy absorbed in the samples. This sample-related loss in counting efficiency is called quenching.

For example, variations in sample color introduce different levels of color quenching. Similarly differences in chlorinated hydrocarbon levels in samples mean varying levels of chemical quenching.
The effect of color or chemical quenching needs to be corrected at the same time as the correction for detector efficiency. Since the correction is handled as a single process, the term quench correction (or quench correction protocol) is used to refer to the whole process, and not just to the correction for quench itself.

Quench correction protocol =
Correction for quench + Detector normalization + DPM correction

**Correction for quenching itself**
To correct for quenching, a number of samples with the same activity but differing quench have to be counted in one detector. This establishes a relationship between a quantity called SQP(I) or AQP(I) and efficiency. SQP(I) or AQP(I) (henceforth referred to as SQP(I)/AQP(I)) is a measure of the isotope spectrum and varies as the quench changes. Once this relationship between SQP(I)/AQP(I) and efficiency is known it is possible to calculate the counting efficiency for any measured SQP(I)/AQP(I) value in the range covered by the measurement of the standard samples. Knowing the efficiency, the DPM can be calculated.

**The quench correction protocol**
However closely matched detectors are, there will be some difference in their counting efficiency. A correction must be made for these differences. That is why the normalization samples are included in the quench correction protocols. The absolute DPM level of the
quench correction samples is given in the protocol to enable DPM calculation of samples.

We first measure a maximum of 12 standards with defined activity and increasing quench level with detector 1 and then count two normalization standards with every detector. The two normalization standards should be representative of the range covered by the standards, i.e. they could be replicates of the first and last standards. Also, the quench level range of all standards should cover the range of unknown samples. The quench standard measurement produces a curve of efficiency versus the quench parameter, SQP(I)/AQP(I) with as many points as there are standardization samples. Differences in the value for SQP(I)/AQP(I) for each detector are also recorded for the two normalization standards.

The activity used should be e.g. 20 μL of tritiated water or some other suitable source giving about 200 000 DPM per well so that the counting time required to get an acceptable precision (e.g. 0.5%) can be about 1 minute. If you do not mind using a longer counting time you can use lower activities.

*Note!* The total time for a quench correction with 200,000 DPM activity would be less than 20 minute whereas for 20,000 DPM, 3 hours would be needed to get the same precision.

It is preferable that about the same number of counts should be collected from each standard in order to get a curve with uniform precision.
The precision requirement is specified during the quench correction protocol setup.

*Note!* When specifying precision it is also important to make sure that the counting time is sufficiently long, so that the precision requirement (and not the counting time) will terminate counting.

**How unknown DPM samples are evaluated**

The actual sequence of events for counting unknown DPM samples is as follows:

1. The SQP(I)/AQP(I) value and CPM are obtained with any detector.

2. The SQP(I)/AQP(I)) value is corrected to be the value it would have been if the sample had been counted in detector 1.

3. The corrected SQP(I)/AQP(I) is used to read off the efficiency from the quench curve made for samples counted with detector 1.

4. The measured CPM value is then divided by the actual detector efficiency to obtain the activity in DPM.

**Preparing single label quench correction samples**

2 to 12 standards are placed in positions A1 to A12 in a 96 or 384-well plate or in positions A1 to B6 in a 24-well plate. No empty positions are allowed at the beginning or between samples. Standards are also placed as follows:
Preparing dual label quench correction samples
For dual label standardization two plates are used and the higher energy nuclide samples are counted first. A maximum of 6 standards can be used on each plate.

<table>
<thead>
<tr>
<th>96-well</th>
<th>96-well</th>
<th>24-well</th>
<th>384-well</th>
<th>384-well</th>
</tr>
</thead>
<tbody>
<tr>
<td>(12 det.)</td>
<td></td>
<td></td>
<td>(12 det.)</td>
<td></td>
</tr>
<tr>
<td>First standard</td>
<td>H11</td>
<td>G11</td>
<td>D5</td>
<td>P23</td>
</tr>
<tr>
<td>Second standard</td>
<td>H12</td>
<td>G12</td>
<td>D6</td>
<td>P24</td>
</tr>
</tbody>
</table>

Quench series

<table>
<thead>
<tr>
<th>96-well</th>
<th>96-well</th>
<th>24-well</th>
</tr>
</thead>
<tbody>
<tr>
<td>(12 det.)</td>
<td>(12 det.)</td>
<td>A1-A6</td>
</tr>
<tr>
<td>First standard</td>
<td>G9</td>
<td>G10</td>
</tr>
<tr>
<td>Second standard</td>
<td>G12</td>
<td>G12</td>
</tr>
</tbody>
</table>

Correction protocols
Within the detector normalization protocol group and the quench correction protocol group each of the protocols is identified by name and by a number from 0 to 99. Protocol number 0 is always the default protocol.

Note! Factory pre-installed detector normalization and quench correction protocols are password protected. Nuclide activities cannot be changed without use of this password, which is initially set to be PerkinElmer.
How to create correction protocols

There are two ways to create new detector normalization and quench correction protocols. One way is to do it from the Edit Counting protocol dialog for the counting protocol you are working with.

When they are created in this way, the parameters of the counting protocol are used as default values for the new detector normalization or quench correction protocol. This saves you time, and is the recommended way.

1. Click **New** under the Detector normalization or Quench correction field
Note! The name of the field depends on whether quench correction is **Off** or **On**.

2. Jump forward to the picture after point 5 on page 65
   The alternative method is as follows:
   1. Open the Protocol groups dialog
   2. Select the Detector normalizations group for a detector normalization protocol or **Quench corrections** group for a quench correction protocol
   3. Click **Open**
4. Click **New** in the Detector normalization (or Quench correction) protocol group window.

A small dialog is displayed with label selections and sample type options.

5. When you have made the selection here, click **OK**
This will lead to the appropriate protocol editor where you can supply the rest of the parameters. The dialog is similar to that dealt with earlier (see page 35). The different parameters for the 4 or 5 panes are described in the Help.

**How to save correction protocols**

When you try to save a detector normalization or quench correction that is not preceded by "-", the warning message shown here will appear.

![Warning message](image)

This reminds you that saved data with this name will be destroyed and you will have to do a new detector normalization or quench correction.

1. **Click Yes** for this to happen

If the protocol name was preceded by "n" or "c" it will be replaced by "_" to show that there are no detector normalization/quench correction data.

**Counting procedure for correction samples**

The plate with the detector normalization/quench correction samples is placed in a cassette which is identified with the corresponding detector normalization/quench correction protocol number in the protocol number area and with a NORM/QCOR label in the function code area.
Note! If the **Background sample** checkbox is checked, a separate empty background plate should be counted before the detector normalization/quench correction plate.

1. Insert the cassette into the rack and close the door
2. Start counting either automatically or manually as described under **Operation** on page 89

The detector normalization/quench correction cassette can be placed anywhere in the rack.

The counting results for the detector normalization/quench correction are output as specified in the protocol, and after them the background and detector efficiency values are given.

*Note! In dual label CPM counting detector normalization and unknown samples should have the same quench level.*

**Results of detector normalization/quench corrections**

When a detector normalization/quench correction is done the results are stored with the detector normalization/quench correction protocol. The data can be used by one or several assay protocols and is selected when editing the protocol.

*Note! You only need to do detector normalization or quench correction *once* for each type of nuclide, sample type and measurement that you are using. After that you just make use of the stored results.*
Crosstalk correction

Crosstalk is a situation in which light pulses from adjacent samples interfere with the pulses of the sample under measurement. This may occur in the case of microplates not designed for liquid scintillation counting. Plates without optical crosstalk and ideal for liquid scintillation counting include the PerkinElmer 1450-401 or 1450-402 Sample Plate as well as the Isoplate™ range. Crosstalk correction is not required and should not be used with these plates.

Crosstalk can be corrected with the MicroBeta² program in connection with detector normalization. Before counting the actual samples, the amount and type of crosstalk is determined using detector normalization samples, with the type of solution as similar as possible to the actual samples.

The detector normalization samples are measured first, and from the results crosstalk factors are corrected for each well position. The calculated crosstalk factors are then used when counting the actual samples.

How to perform detector normalization with crosstalk correction

For this process you need sample solution with some activity (+ scintillant), which corresponds to that of the samples to be analyzed, i.e. it has the same radionuclide and solvent. You also need background solution, which is like the sample solution, but without the activity. For luminescence crosstalk correction, the sample is a high signal sample and background is a blank sample.
Note! Use sufficiently high activity samples in normalization for there to be crosstalk, but do not exceed 10,000,000 CPS in luminescence or 1,000,000 CPM in LSC.

1. Pipette the same volume as in the samples, into well D6 (24-well plate), G11 (96-well plate), N22 (384-well, 6 detectors) or M21 (384-well, 12 detectors).

2. For a 24-well plate, pipette background solution into wells A1, C6 and D5. D5 is only needed for strip plates and A1 if background correction has been selected.

For a 96-well plate with a 1-6 detector counter, pipette background solution into wells A1, F11 and G10. G10 is only needed for strip plates and A1 if background correction has been selected.

With a 12-detector counter, use positions A1 and F11. Strip plates may not be counted.

<table>
<thead>
<tr>
<th>Normalization sample positions on a plate</th>
<th>24-well</th>
<th>96-well</th>
<th>96-well (12 det.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample solution</td>
<td>D6</td>
<td>G11</td>
<td>G11</td>
</tr>
<tr>
<td>Background</td>
<td>A1</td>
<td>A1</td>
<td>A1</td>
</tr>
<tr>
<td>Crosstalk solution (Strip plates only)</td>
<td>C6</td>
<td>F11</td>
<td>F11</td>
</tr>
<tr>
<td></td>
<td>D5</td>
<td>G10</td>
<td>*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>384-well (12 det.)</th>
<th>384-well (12 det.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample solution</td>
<td>N22</td>
</tr>
<tr>
<td>Background</td>
<td>A1</td>
</tr>
<tr>
<td>Crosstalk solution (Strip plates only)</td>
<td>M22</td>
</tr>
<tr>
<td></td>
<td>N21</td>
</tr>
</tbody>
</table>

*Strip plate not available
For a 384-well plate with a 6-detector counter use positions A1 and M22, and N21 for strip plates.

With a 12-detector counter, use positions A1 and M22. Strip plates may not be counted.

3. Close the sample plate with a sealing tape and place it in a 1450-102 (24-sample), 1450-105 (96-sample) or 1450-130 (384-sample) cassette for counting.

4. Check the **Crosstalk correction** checkbox in the protocol editing box (see page 66)

![Edit detector normalization protocol](image)

5. Start the detector normalization run as described earlier

**Luminescence counting**

Microplates of the PerkinElmer OptiPlate™ range are recommended for luminescence applications. With MicroBeta² instruments, luminescence assays on, for example, nylon membranes can also be directly quantified.

When the Luminescence option is installed it allows the temperature of the upper detectors to be reduced and stabilized. You can enable detector cooling and set the
target temperature by means of system settings (see page 107).

Due to the high signal, luminescence reactions can be measured using single PMTs (photomultiplier tubes). The traditional microtitration plates are totally opaque (black or white). These plates are counted with the upper tube and a 1450-105 cassette is used. The filters are counted with the upper or lower tube and a 1450-104 filter cassette is used.

When intense luminescence samples on filters are counted with the upper tube only, a 1450-111 luminescence cassette should be used. This prevents saturation of PMTs and crosstalk between adjacent positions is minimized by cutting the intensity of the luminescence light.

The luminescence units used are CCPS, corrected counts per second. Before counting the actual samples, a detector normalization should be performed using a luminescence solution as similar to the actual samples as possible.

**Creating a luminescence detector normalization protocol**

Creating a detector normalization protocol is done in the same way as creating other detector normalizations.

1. Select the Detector normalizations group and then **New**
2. From the list of nuclides select "Lumin" as shown below
Corrections

Other parameters can be set in the normal way, but see also page 73 for settings specific for injector counting.

**Preparation of normalization samples (without JET)**

You need to make a sample solution that corresponds to the samples to be analyzed, i.e. has the same enzyme and substrate.

1. Pipette the same volume as in the samples, into well/membrane position D5 (1-6 detector 24-wells), G11 (1-6 detector. 96-wells), G11 (12-detector 96-wells), N22 (6-detector 384-wells) or M22 (12-detector 384-wells).

| Luminescence normalization sample positions on a plate |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| 24-well                         | 96-well         | 96-well         | 384-well        | 384-well        |
| (12 det.)                       | (6 det)         | (12 det.)       | (12 det.)       | (12 det.)       |
| Sample solution                 | D5              | G11             | G11             | N22             | M22             |
An empty plate is used for background measurement.

*Note!* For 24-well plates use plate type 1450-402 and cassette type 1450-102 when setting up the luminescence normalization protocol parameters.

2. If the samples are on a microplate, close it with 1450-461 sealing tape and place it on a 1450-105 cassette for counting.
   
   If they are on a filter, enclose it in a 1450-432 plastic sample bag and place it on a 1450-104 filter cassette or on a 1450-111 luminescence filter cassette.

**Preparation of normalization samples (with injectors)**

With flash luminescence assays, normalization cannot be performed by measuring the same samples in all of the detectors. Instead normalization samples are needed for each detector, and in order to ensure good normalization results, the use of several replicates is recommended.

The plate map information presented below, including the positions on each type of plate for the number of replicates you wish to use, can be also obtained directly by generating plate maps with the Workstation software (see page 77).

**Luminescence plate maps for 96-well formats**

The plate maps for different detector configurations with 96-well plates and the maximum number of replicates are shown below. If there is less than the maximum number, the replicates are set from left-to-right and top-to-bottom.
### 12-detector models without background samples

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### 12-detector models with background samples

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1- and 2-detector models without background samples
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1- and 2-detector models with background samples
Rows E-F are empty in the 1-detector model.

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Luminescence plate maps for 384-well formats

The corresponding blocks for the maximum number of normalization sample replicates and the positions for background samples when using 384-well formats are as follows:

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<th>Blocks for normalization samples</th>
<th>Background samples, if used</th>
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<td>from A19 to D24</td>
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<td>from G7 to J12</td>
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<td></td>
<td>from G13 to J18</td>
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<td></td>
<td>from G19 to J24</td>
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<td></td>
<td>from M1 to P6</td>
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<td>from M7 to P12</td>
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<td>from M13 to P18</td>
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<td></td>
<td>from M19 to P24</td>
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</tr>
<tr>
<td>6</td>
<td>from A1 to D8 plus E1 to E4</td>
<td>A1, I1, A9, I9, A17 and I17</td>
</tr>
<tr>
<td></td>
<td>from I1 to L8 plus M1 to M4</td>
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<tr>
<td></td>
<td>from A9 to D16 plus E9 to E12</td>
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<td>from I9 to L16 plus M9 to M12</td>
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<td>from A17 to D24 plus E17 to E20</td>
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<tr>
<td></td>
<td>from I17 to L24 plus M17 to M20</td>
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</table>

Luminescence plate maps for 24-well formats

Plate maps for different detector configurations with 24-well plates and the maximum number of replicates are shown below. If there is less than the maximum number of replicates, the replicates positions are from left-to-right and top-to-bottom.
6-detector models without background samples

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<tbody>
<tr>
<td>A</td>
<td>S1</td>
<td>S1</td>
<td>S2</td>
<td>S2</td>
<td>S3</td>
<td>S3</td>
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<tr>
<td>B</td>
<td>S1</td>
<td>S1</td>
<td>S2</td>
<td>S2</td>
<td>S3</td>
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<tr>
<td>C</td>
<td>S4</td>
<td>S4</td>
<td>S5</td>
<td>S5</td>
<td>S6</td>
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<tr>
<td>D</td>
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6-detector models with background samples

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<tr>
<td>A</td>
<td>B1</td>
<td>S1</td>
<td>B2</td>
<td>S2</td>
<td>B3</td>
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<td>B</td>
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<td>S2</td>
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<td>S3</td>
<td>S3</td>
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<tr>
<td>C</td>
<td>B4</td>
<td>S4</td>
<td>B5</td>
<td>S5</td>
<td>B6</td>
<td>S6</td>
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<td>D</td>
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</table>

1- and 2-detector models without background samples
Row C is empty in the 1-detector model.

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<tr>
<th></th>
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<tbody>
<tr>
<td>A</td>
<td>S1</td>
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<tr>
<td>C</td>
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1- and 2-detector models with background samples
Row C is empty in the 1-detector model.

<table>
<thead>
<tr>
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<td>A</td>
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<tr>
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<td>B2</td>
<td>S2</td>
<td>S2</td>
<td>S2</td>
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<tr>
<td>D</td>
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</tbody>
</table>

How to generate and view normalization plate maps
LumiJET normalization plate maps such as the ones shown above can also be viewed on screen.
1. When setting up the luminescence normalization protocol click the JET tab

2. Make sure that the **Use Injector module(s)** option is selected

3. Specify the number of replicates

The software will not allow you to choose more than the maximum permitted amount.

4. Make other settings as required and click **OK**
The picture above is an example of a plate map for a 384-well plate counted on a 6-detector counter with background correction. The background samples are represented as the black rings. For each detector 15 replicates (the red rings) are being used.

**Luminescence normalization counting procedure**

The plate with the luminescence normalization samples is placed in a cassette which is identified with the corresponding normalization protocol number in the protocol number area and with a NORM/QCOR label in the function code area.

1. Insert the cassette into the rack and close the door
2. Start counting either automatically or manually as described under **Operation** on page 89

The detector normalization cassette can be placed anywhere in the rack.

The counting results for the detector normalization are output as specified in the protocol, and after them the background and detector efficiency values are given.
Operation
Operation

Coding cassettes and filtermats

Cassette code system
Barcode labels are used for cassette and sample recognition. They are attached to the marked area of the ID support plate. This is a separate piece of plastic which can be clipped onto the appropriate area of a cassette as shown in the figure. When fixing ID labels on the ID support plate, take care that the fixing area is clean. If the protocol ID label is dirty or placed the wrong way round, the barcode reader may fail to read it. In this case the default protocol (prot.No. 0) is used.

Cassette codes used
An ID support plate has four fields (areas) to which a barcode can be fixed. The meaning of each field is described below. The figure shows a counting cassette. The cassette number is 1 and the counting protocol to be used is 60.

Function codes
The FUNC field can be labeled as follows:
No label - The cassette is a normal sample cassette without any special function

QCOR - The cassette is to be used for quench correction. The number of the quench correction protocol is given in the PROT field.

NORM - The cassette is to be used for detector normalization. The number of the normalization protocol is given in the PROT field.

STOP - counting will stop after this cassette has been counted.

**Cassette number**
This is a simple number in the range 0-99 and can be output with the results.

**Protocol number**
This is normally a simple number in the range 0-99 (for the exception see **Stop cassette** below). The type of protocol it refers to depends on what is specified in the FUNC field. If nothing is specified in the FUNC field then it is the number of a counting protocol.

**Assay protocol number**
This field is available as an additional identifier for output data.
Stop cassette

If you fix a STOP code to the last cassette it means that this cassette will be the last one counted. However it can be tedious to remove this STOP code and fix it to another cassette when more cassettes are loaded. To avoid this an empty "stop cassette" can be used. Fix a STOP code on both the protocol and function code field of an empty cassette and put that cassette after the last cassette to be counted.

Warning! A STOP cassette must have two STOP codes on it. If you only put one on an empty cassette then you waste time counting it and in the worst case you may inject liquid when there is no plate. To avoid the possibility of this see the appendix.

Cassette operation

If MicroBeta® is loaded with many sample cassettes that are to be counted with different protocols, the cassettes are barcoded with the respective protocol number. If there is no protocol number on the cassette, the cassette is counted with the same protocol as the previous one i.e. it is considered to be part of the same assay. Only the first plate of an assay should be barcoded. Each new barcode found starts a new assay. To stop the counting automatically, the STOP code should be used.
Filtermat coding

On the lower edge of the filtermat there are two groups of twelve small circles (see the figure on the next page). They are for marking the date. This is done by cutting or clipping off appropriate circles. The first twelve circles are to specify the day and the second twelve the month. In the figure below the leftmost 9 circles stand for the numbers from 1 to 9 and the next three circles for 10, 20 and 30 respectively. E.g. the 25th day is marked by cutting off the 20 circle (the 11th circle from the left) and the 5 circle.

The month is marked using the rightmost 12 circles, the first circle corresponding to January, the second to February etc.

The row of 7 small circles on the right-hand edge (next to sample column A12 to H12) is used for sample
identification. The circles stand for the sequence 1, 2, 4, 8, 16, 32, 64. By cutting off appropriate combinations of circles the filtermat can be marked with a number in the range 1 to 127.

**Plate ID reader**

If the optional plate ID reader is installed then it is possible to read barcode IDs from plates directly. The Plate ID must consist of 7-bit printable ASCII characters and spaces. The following barcodes are supported: Codabar, Code 39, Code 128, Interleaved 2 of 5, UPL and EAN. If this is installed it will be shown in System information.
**Loading cassettes**

The cassette rack is in its middle position when being loaded. A motor moves the rack up or down one step at a time.

Cassettes should be loaded into the rack with the sample plate upwards and the ID labels towards you.

1. Open the door on the front of the counter

2. Load cassettes into the rack with the sample plate upwards and the cassette ID labels towards you.

3. Place the cassette to be counted first on the bottom shelf, numbered 1, and remaining cassettes in the order that they are to be counted on shelves 2 to 16.

Empty shelves between cassettes are allowed, and the first cassette need not necessary be on shelf 1 as long as there is no other cassette below it.

4. After loading, close the door
Starting counting

Automatic start
When barcode labeled cassettes are used, you can use Automatic start.

1. Click the **Start** button in the main window.

If Automatic Start Confirmation is selected in Options, a Confirm Automatic Start dialog will appear.

2. Click **OK** to start the counter in automatic counting mode with the live display maximized while counting is going on.

   Alternatively click **Cancel** to cancel starting.

   If the check box "Do not show this confirmation again" is checked when **OK** is pressed, the Confirm Automatic Start
dialog is not shown when **Start** is pressed next time. You can restore this confirmation in the Confirmations dialog in System Settings (see page 107).

**Manual start**
If cassettes are not barcoded you need to start the counting manually.

1. Select a protocol and click the **Start** button

The Manual start dialog will appear. This shows the basic parameters of the selected assay protocol and has fields for the shelf number, delayed start and naming and path for output file.

![Manual Start Dialog](image)

*Note!* In the Enhanced Security mode the **Path** field and **Browse** button to its right are disabled.

2. Edit or accept the parameters in the dialog
Note! The file output editing in the manual start window overrides the output file name specified in the output settings of the protocol. The value is taken from the current **File1** tab as default (see page 49). The name can be changed and it will be used for the next assay started with the **Start** button of the **Manual Start** dialog. The value entered is not saved in the protocol.

**Note!** Counting (delayed or normal) can be started by clicking the **Start** button in the manual start dialog. If delayed start is chosen, you can give the delay time in minutes. After clicking the **Start** button, a floating **MicroBeta²** - Waiting window will be shown. The window shows the time left before the start of counting and has two buttons: **Start now** and **Abort**. Counting can be started any time by clicking the **Start now** button. The **Abort** button will cancel starting.

3. Click the **Start** button

**Stopping counting**

Once counting has been started the **Stop** button and **Stop** item in the Counter menu will be activated.

1. To stop counting, click the **Stop** button. You will be asked if you really want to stop counting (during this time counting will be continuing).

2. Click **OK** to confirm. Otherwise click **Cancel** to continue counting.
Loading new samples during counting

The door on the front of MicroBeta\(^2\) is automatically locked during counting. You can, however, interrupt the counting. The door lock is then released and new samples may be loaded.

Once counting has been started the **Pause** button and **Pause** item in the Counter menu will be activated.

1. To pause counting, so that the counter door lock is released, click the **Pause** button.

You can then open the counter door and access the sample compartment.

Clicking **Pause** also causes the **Start** button to become the **Continue** button

2. When you are ready to resume counting at the point where you left off, click the **Continue** button.
Live display

During counting the live display shows the situation in graphical format and allows you to monitor the counting. All plates of the currently (or most recently) measured assay protocol can be browsed and individual CPM values of measured wells can be viewed.

Opening and closing

The live display is automatically created and minimized when the program is started and it cannot be closed. Selecting Close from the system menu only minimizes it.

1. To maximize live display click the Live icon or Live item in the View menu

Live display can be sized (but not below its minimum size) as you like. When counting starts, it is automatically maximized. When counting ends, its size is restored.

Mode

The live display can be in three modes: Dot, Numeric or Spectrum. The Mode field at the upper left corner of the live display allows you to select the mode by clicking the appropriate button: Numeric, Dot or Spectrum:
Dot mode

In Dot mode, CPM values are shown as colored dots superimposed on a picture of the plate.
**Numeric mode**

In Numeric mode, values are shown as numbers written on the well positions.
**Spectrum mode**

In Spectrum mode a spectrum is shown for a selected detector.

The mode will also show live kinetics (repeats) of LumiJET Luminometry measurements.
Viewing results
Introduction

The right side of the protocol group window is for result handling. The "Results of" list contains the names and dates of the results files of the assay protocol. New results appear at the end of the list when the assay is measured.

You can maximize the window, and adjust the size of the fields to show more of the name or path or date as required. Use the mouse to drag the dividing line between the column headings to make the field the size you want.
The contents of the results list changes when you change the assay protocol selection.

In the protocol group window there are three buttons that allow result file handling.

**Result operations**

You can **Copy** a result, **Delete** it or **Open** it by clicking the appropriate button. You can use a built-in viewer or Excel (if it is installed) to open a file. Which program is started depends on the extension (.txt or .xls respectively). You can define which ASCII viewer is started for txt files. The default is a viewer program supplied with the workstation called (WIWView). An alternative viewer can be defined via the Viewer button on the Settings box, (see page 107).

For viewing with other programs, please refer to the instructions supplied by the supplier of the program.

WIWView allows you to view and print result files. It supports four different views of the data:

The **Text view** shows the contents of the file as text.

The **Plate view** shows graphical plate images.

The **Curve view** shows curves.

The **Raw Text view** shows the whole contents of the file as text.
Results button

Clicking the **Results** button on the main workstation toolbar opens a Results dialog. The dialog has a list box containing all the results file names. It also has the buttons **Open**, **Copy**, **Delete**, **Goto group** and **Close**.

![Results dialog](image)

**Open**, **Copy** and **Delete** work exactly the same as in the protocol group window described above. Double-clicking on the results file has the same effect as clicking **Open**.

Clicking the **Goto group** button will open the corresponding assay group window and select the assay and result.
Note! In the Enhanced Security mode the **Delete** button is only enabled for users who belong to the WIW Editor group. The **Copy** button is enabled for users who belong to the WIW User or WIW Editor group and there are no protocols running.
System operations
System operations

When you click System (the button or the item in the File menu) a dialog opens with three buttons allowing you to select System view, Settings or System information.

Note: in the Enhanced Security mode the System view button is enabled only for a user belonging to the WIW System Manager group and the Settings button is enabled only for users belonging to the WIW Administrator, WIW System Manager or WIW Auditor groups.

System view

You can view and modify the objects in the program through the System view. Click the System button on the toolbar or select System from the File menu to get the System setup dialog [A]. Click the System View button. A new dialog will open with a list of different items (protocols, samples etc.) that you can modify [B].

You can select any of these items and click Modify to see a list of all the items of that type (e.g. all plates and filters). Beside the list are command buttons for the operations: Edit, Copy, Delete and New.
1. Click, for example, **New** to add a new item within the selected category [D].
Note! Some buttons may be disabled, because that particular operation is not allowed for the item in question.

Settings

If you click the System button or select System from the File menu and then click the Settings button, the settings dialog is displayed. It has eleven buttons, Counter, Results, Viewer, MultiCalc§, Confirmations, Options, User*, Audit Trail*, Security* and File* plus Close.

The function of each of these buttons is described in the on-line help but note the following:

§ MultiCalc is not enabled in the Enhanced Security mode.
*The following buttons are only enabled in the Enhanced Security mode subject to the following restrictions:
User is only for the use of the WIW Administrator.
Audit Trail is only for a member of the WIW Auditor group.

All the other buttons are enabled only for a user who belongs to the WIW System Manager group.
System information

The system information window shows the settings and features of the counter. The system information is queried from the counter when the workstation is started if the counter is in the ready state.

Robotic loader interface

This model of MicroBeta\(^2\) is designed to enable a robotic loader to be used. There is one shelf instead of the usual 16 or 32 shelves in the cassette rack. This shelf protrudes from the instrument. The robotic arm can place a plate on this shelf or remove one from it. When a plate has been placed onto the shelf it is then taken into MicroBeta\(^2\) for counting. The plate orientation should be defined on the Protocol editor Counting control page, see page 35, because the
orientation of the plate when it is taken into the counter can be different from the normal one.

**Enable IPA**

You must click here if you want the IPA protocol to be enabled. If this item is gray then it means the IPA protocol has already been selected. See the chapter on IPA for more details.

**Exiting from Windows Workstation**

1. To exit from the program, select **File** on the menu bar and then click **Exit**.

2. Confirm that you want to exit by clicking **Yes** or cancel exiting by clicking **No**.
Instrument Performance Assessment (IPA)
Instrument Performance Assessment (IPA)

The Instrument Performance Assessment (IPA) feature enables you to periodically monitor the twin tube detector coincidence efficiency and background in time for tritium and carbon-14 nuclides in MicroBeta². Results of multiple runs can be viewed and printed as a graphical trend or numerical report.

IPA plates

Two plates are needed for running a IPA protocol: a blank black background plate followed by a Wallac 1450-471 96-well normalization plate.

IPA group and IPA protocol

The IPA group and protocol is created by selecting Enable IPA from the File menu. This will cause the IPA group window to open as shown in the figure below. Note: in the Enhanced Security mode Enabling IPA is allowed only for users who belong to the WIW System Manager group. The IPA protocol cannot be created, copied or deleted. The History button opens the Protocol History browser. This History button is enabled only under Enhanced Security mode. There is only one IPA protocol in the group, named IPA.
**Note!** In the Enhanced Security mode the IPA group window has the following restrictions:

The **Start**, **Delete** (results) and **Template** buttons are enabled only for users who belong to the WIW Editor group. The **Copy** (results) button is enabled for users who belong to the WIW User or WIW Editor groups providing that no protocols are currently being run.

Clicking the **Protocol** button allows the protocol to be changed. The following IPA protocol parameters can be edited: Counting time for standards and background, barcode ID, half-life correction, DPM Activities, owner name, next run ID, password, local and validity check (in the Enhanced Security mode this is checked and disabled).
Note: in the Enhanced Security mode the OK button is only enabled for users who belong to the WIW Editor group and the Run ID cannot be modified.

Clicking **Plate map** causes the IPA plate to be displayed. Clicking the **Printout** button shows the printout options.

On the right of the group window is a list of all the results files that have been produced by running the IPA protocol. A **Trend** plot, **Report** and **History Report** of the results can be viewed by clicking the **IPA view** button. Results can be deleted using **Delete** button.

*Caution! The deletion operation cannot be undone.*

**Running IPA**

The IPA protocol can be run in automatic mode with barcoded plates or it can be started manually. If you use a barcode you must select the correct bar code ID (the default is 99 although this can be changed). When the protocol has been edited as required press the **Start** button on the toolbar. The first cassette bar code must have NORM in the FUNC field and the second cassette must have STOP in the FUNC field.

Manual start is done by pressing the **Start** button in the IPA group window.
IPA View

Results from the run of the IPA assay can be accessed only with the IPA View. There are no other output files from the IPA protocol. If there are any errors during the IPA run, the results are not saved. This IPA view has three pages: Trend, Report and History Report.

Trend Page

The Trend page shows the selected result item as a trend for the selected detector.

Printing Trends

IPA trends can be printed by pressing the Print button on the toolbar while the Trend page is active. The dialog has checkboxes for each label and for each of the items. There is also a Detector drop-down list box which has all
detector numbers and a special **All** selection. There is also a checkbox **Print detectors separately**.

![IPA Trend Print Options dialog](image)

When the **IPA Trend Print Options** dialog is opened, the settings are such that the current contents of the trend page will be printed. Clicking **OK** will show the standard windows print dialog.

As a minimum, the printout contains one graph, single label, single item and single detector.

As a maximum, the printout contains 96 graphs (12 detectors * 4 items * 2 labels).

Two graphs are printed on the each page. The order of the output is first grouped by detector, then by label and last by item.

**Report page**

The report page shows single results in table format. Each label has a separate table. The items are the columns of the tables. Tables have as many lines as there are detectors in the counter. The report can be printed by clicking the **Print** button.
When **IPA View** is opened, the Report tab shows the result that was selected in the result list of the IPA group. The result can be selected from the **Result** drop-down list above the report.

**History Report tab**

The report tab shows the history of each item as a table where detectors are columns and dates and times are rows. Report can be printed by clicking the **Print** button.
Appendix A
Error messages
Error messages

Error messages may sometimes appear; the following error messages (in block letters) are explained below:

PROGRAM NOT FOUND
The viewer program is not found. You need to check the System Settings and verify that it is specified there.

PROGRAM EXECUTION FAILED
The Viewer/Excel program is found but could not be executed. You need to verify the Viewer/Excel installation.

RESULT FILE DELETED
This message comes when you try to operate on a result file that has been deleted.

CANNOT DELETE COUNTING
You tried to delete a counter protocol that is being counted.

RESULT FILE NOT DELETED
This comes when the result file could not be deleted because it has already been deleted externally to the MicroBeta² Windows Workstation program. The name is still on the list and the program is asking if you want the name to be deleted from the list.

DELETE NORM/QCOR DATA
When you save a normalization/standardization you are warned that the existing normalization/standardization data will be deleted.
MASTER OR RESTRICTIONS EDITED
This appears when you go to counter parameter defaults after editing master plates or restrictions and warns you that the editing is effective as a default value only after the template has been saved.

LABELCOUNT CONFLICT
This comes when you try to enter a protocol that has illegal label or single/dual values that conflict with the template restrictions. The single/dual setting and labels are set to the default values. If you do not want to change settings, cancel protocol editing.

FILE NOT ASSOCIATED
This comes when the result file was opened but there was no association with the extension
- maybe because Excel was not installed
- some other extension than xlw or xls was selected for the output file.

WARNING IN USE
This tells you that you are trying to remove a normalization/standardization that is used by other protocols.

TOO MANY DISPLAY/PRINT ITEMS
Too many items were added to the list of display or print items.
CANNOT EXIT WINDOWS
You tried to shut down Windows while dialogs were open in MicroBeta\textsuperscript{2} Windows Workstation.
Appendix B
Pre-installed protocols
Pre-installed protocols

Pre-installed protocols supplied with MicroBeta\textsuperscript{2} counters include counting protocols, detector normalization protocols and quench correction protocols as listed below.

All protocols are password protected, and on instruments leaving the PerkinElmer factory the password for editing pre-installed protocols is \textit{PerkinElmer}.

Supplied with all instruments

Counting protocols

- C-14 Lumaplate
- Cr-51 Liquid Scintillant
- Cr-51 Lumaplate
- H-3 Filtermat - Cell Proliferation - Filtermat
- H-3 Flashplate
- H-3 Liquid Scintillant
- H-3 SPA
- H-3 Unifilter
- Luminescence ATP - Firefly Luciferase

Detector Normalizations

- C-14 Lumaplate
- Cr-51 LSC
- Cr-51 Lumaplate
- H-3 Factory_Preset
- H-3_Topread
- Luminescence_FireflyLuc-ATP_Factory_Preset
Quench Corrections
- H-3 SPA

Additionally for 1 and 2 detector units

Counting protocols
- H-3 Minivials 4mL
- Wipe Test

Additionally for 1 and 2 detector LumiJET units

Counting protocols
- Aequorin single read 96-well

Detector Normalizations
- Luminescence Aequorin JET

Additionally for 1 and 2 detector LumiJET dual dispenser units

Counting protocols
- Aequorin Dual Screen 96-well
- Dual Luciferase 96-well

Detector Normalizations
- Luminescence Aequorin JET
Additionally for 6 and 12 detector LumiJET units

Counting protocols
- Aequorin single read 96-well

Detector Normalizations
- Luminescence Aequorin JET

Additionally for 6 and 12 detector LumiJET dual dispenser units

Counting protocols
- Aequorin Dual Screen 384-well
- Dual Luciferase 96-well

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- Luminescence Aequorin JET
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