and 2450 MicroBeta²™ 2460 MicroBeta² LumiJET

Liquid Scintillation and Luminescence Counters

Valid for MicroBeta² instruments with Workstation software



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Warning

This equipment must be installed and used in accordance with the manufacturer's recommendations. Installation and service must be performed by personnel properly trained and authorized by PerkinElmer Life and Analytical Sciences.

Failure to follow these instructions may invalidate your warranty and/or impair the safe functioning of your equipment.



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Chapter 1 Introduction

Introduction

Guide to the Instrument manual

This manual describes the two versions of the PerkinElmer $MicroBeta^2_{TM}$ microplate scintillation and luminescence counter, 2450 $MicroBeta^2$ and 2460 $MicroBeta^2$ LumiJET. The main difference between the two versions is that $MicroBeta^2$ LumiJET has reagent injectors, and hence supports additional luminescence applications. Both instruments versions are operated with $MicroBeta^2$ Workstation software.

Most of the information provided in this manual relates to both models, where there is a difference, this is explained.

Chapter 2 provides a description of MicroBeta² and its functions.

Chapter 3 provides information on user documentation as well as important warnings for users of the system.

Chapter 4 covers routine maintenance activities that are carried out by the user.

Chapter 5 deals with the calculation methods employed.

Chapter 6 comprises a detailed list of specifications.

Chapter 7 includes important information on waste disposal.

Chapter 8 is a list of abbreviations and acronyms used in the manual or otherwise relating to use of the instrument.

Chapter 9 provides installation information, and is only required at the time of installation

At the end of the manual is an alphabetical index.

Operation of both 2450 MicroBeta² and 2460 MicroBeta² LumiJET is described in the A5-sized User Manual that forms part of the MicroBeta² Workstation software product.

Safety and radioactive materials

The following comments about precautions and safety measures in handling radioactive materials are included as a guide and are not intended to be fully comprehensive. More complete details may be found elsewhere, for example in the booklet SAFE HANDLING OF RADIONUCLIDES, published by the International Atomic Energy Agency, Vienna; this may be recommended as a useful code of practice appropriate to radio-chemical laboratories.

Unless MicroBeta² is installed in a dedicated radionuclide laboratory, limitations should be placed on the amount of active material in the

laboratory area depending on toxicity and type of chemical operation. For high toxicity material and wet chemical operations involving the risk of spillage, the IAEA recommend a maximum activity of about 10 μ Ci.

Personnel should be properly trained in the safe handling of these materials, maximum levels of stored activities should be set, proper records should be kept, and a definite monitoring schedule maintained.

The areas where samples are handled should be kept clean and free of dust. This is most easily accomplished if all surfaces are as smooth as possible and if the minimum number of extra surfaces is introduced into the room. Lastly it is extremely important to store all radioactive materials in a separate room to which access is restricted. Chapter 2 Functional description

Functional description

Liquid scintillation and luminescence counter

With 2450 MicroBeta² you can count beta or gamma emitting samples or glow luminescence samples. With 2460 MicroBeta² LumiJET you can also count flash luminescence samples in which reagent injection is required. In either counter version, you gain the benefit of several powerful counting techniques combined in one counter.

Both MicroBeta² versions handle either 24/96 or 96/384-well microplates, as well as flexible plates, microcentrifuge tubes, filtermats and 4mL liquid scintillation vials. Before counting, the instrument itself determines which type of sample format is being used.

The sample plate or other format is placed in a special cassette for counting, which you can identify with barcoded labels. Load the cassette into the cassette rack behind the door with the plate upwards and the ID labels towards you, then close the door.

The counter is operated through a dedicated system computer running MicroBeta² Windows Workstation software.

Operation

Starting counting

After samples have been loaded, the door of the counter needs to be properly closed. A door lock ensures that the door cannot be accidentally opened while counting is progressing. Counting is started from the Workstation display. The counting may be started automatically by clicking the **Start** button if the cassettes are barcoded. If barcoding is not used, the counting is started manually as described in the User Manual.

While counting is taking place the uncorrected CPM or CPS values are available for viewing on a live display. The values are converted to CCPM1, DPM1 or CCPS1 values when the whole plate has been counted.

Pausing counting

If you would like to check the cassettes or add additional ones, counting must first be paused by clicking the **Pause** button on the Workstation display. After checking or adding the cassettes, counting is restarted by closing the door and clicking **Continue**. Counting of those samples that were in the counting position when the door was opened will be repeated.

Stopping counting

Counting can be stopped at any time by clicking the **Stop** button. The program terminates counting.

Cassette rack and transportation system

The cassette rack is located inside the door and consists of 16 shelves numbered 1 to 16 from the bottom upwards. Twelve-detector versions of 2450 MicroBeta² may alternatively be supplied with 32 shelves. The maximum capacity is 384 samples when using 24-well plates, 1536 (3072 with 32 shelves) samples when using 96-well plates, and 6144 (12288) samples when using 384-well plates.

Any combination of the two types of plates accepted by your $MicroBeta^2$ (96 and 24, or 96 and 384) can be used (see mask plate, below). The type of cassette (384, 96 or 24 position cassette) is recognized automatically by the counter.

Loading

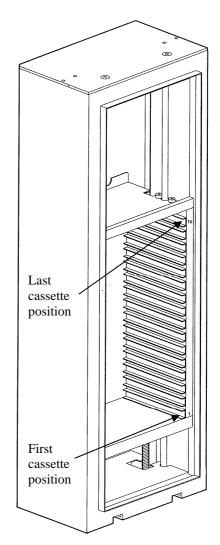
The cassette rack is in its middle position when being loaded. A motor moves the rack up or down one step at a time.

Cassettes should be loaded into the rack with the sample plate upwards and the ID labels towards you. The cassette to be counted first should be inserted on the bottom shelf, numbered with 1, and the next cassettes on the following shelves. Empty shelves between cassettes are allowed, and the first cassette must not necessary be on shelf no. 1 as long as there is no other cassette below it.

After loading, close the door properly.

Processing

During processing, one cassette at a time is moved to the transportation system. This is an X-Y transfer mechanism, which first moves it to the ID reader for reading of the barcodes on the cassettes. Then it is moved to the first counting position. After a cassette has been counted, it is moved back to the rack. The elevator moves the cassette rack down one position and the next cassette is moved to the transportation system.



The movement of the cassette rack, and cassettes on the transportation system normally occurs automatically without any need for assistance from the user. However, manual operation is also possible.

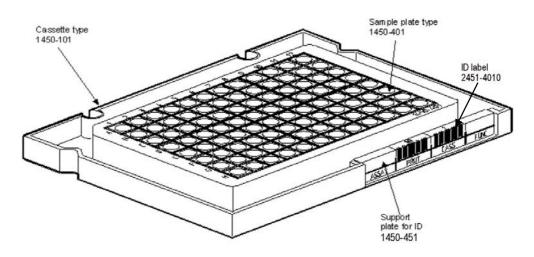
Note! Do not move the rack up or down when a cassette is in the counting position. Always first clear the conveyor. Re-start the system if the rack and transportation system becomes blocked.

During counting the door lock can only be released by pausing counting, which will cut off the high voltage in order to protect the detectors.

Cassettes

A MicroBeta² cassette is designed to hold a microplate or filter and to allow it to be moved from the rack to the conveyor and back again. Cassettes that can be used in MicroBeta², cover 24, 96 and 384 sample formats. Special adapters and inserts are used with cassettes to permit counting of Eppendorf tubes (or equivalent) and 24 well culture plates. See page 61 for details of the various cassettes, adapters and inserts used.

If the instrument has six or less detectors it can take either a 96 or 24 well cassette or a 96 or 384-well cassette depending on the mask plate used (see page 13). If it has twelve detectors then only 96-well and 384-well cassettes can be used.



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. Sometimes it happens that the protocol ID label is dirty or placed the wrong way round and the barcode reader fails 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 DPM quench correction (see page 16). The number of the quench correction protocol is given in the PROT field.

NORM - The cassette is to be used for CPM detector normalization (see page 16). The number of the detector normalization protocol is given in the PROT field.

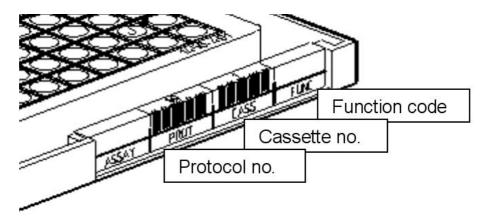
STOP - counting will stop after this cassette has been counted.

Cassette number

This is a simple number in the range 0-99 and can be output with the results. If the function code is also a number then 100*cassette number + function code is output (in the range 0..9999).

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, this cassette will be counted before counting terminates. However it can be troublesome to remove and refasten STOP codes when additional cassettes are loaded. To avoid this additional work, a stop cassette can be used. Stick 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.

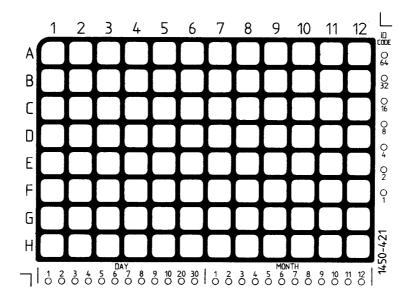
Cassette operation

If MicroBeta² is loaded with many sample cassettes that are to be counted with different protocols, the cassettes are coded 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. 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 following figure). 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 above, 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.

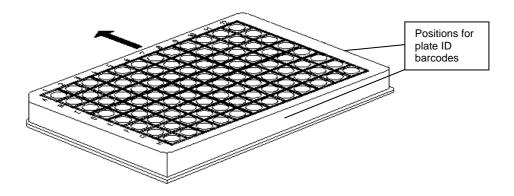
The month is marked using the rightmost 12 circles, the first circle corresponding to January, the second February etc.



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.

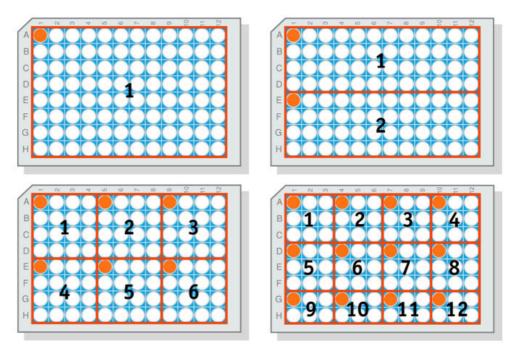
Plate ID reader

If the optional plate ID reader is installed then it is possible to read barcode IDs from plates. The following barcodes are supported: Codabar, Code 39, Code 128, Interleaved 2 of 5, UPL and EAN.



Detectors

MicroBeta² instruments are supplied with one, two, six or twelve pairs of photomultiplier tubes. The detectors are placed in a 1x1, 2x1, 2x3 or 3x4 formation respectively.



The tubes of one pair are situated on opposite sides of the counting block, one above and one below the block. Each pair operates in coincidence mode, which greatly reduces single photon background events. The out-of-coincidence signal is also measured simultaneously.

When a cassette moves into the counting position the labels on the cassette are read by the barcode reader. Then 1, 2, 6 or 12 samples on the cassette are counted simultaneously according to the protocol specified by the label on the cassette.

Due to the relatively small sample volume there is such a low background count that no extra lead shielding is needed, thus reducing the weight of the instrument.

To avoid ambient light affecting the photomultiplier tubes, the inside of the instrument is painted black and the door has a light seal along its edge.

To protect the detectors, the high voltage of the photomultiplier tubes is cut off when the door is open. A warning message is displayed if counting is attempted while the door is not properly closed.

Mask plate

The mask plate is an aperture plate situated within the detector block. The mask plate has holes to suit two alternative plate formats. By default the mask plate for 1- and 2-detector models suits either 24 or 96-well formats, while for 6- and 12-detector models a 96 or 384-well mask plate is default. As an option, a 24/96 mask may be supplied with 6-detector models instead of the standard 96/384 mask.

One and two detector models

In MicroBeta² models with 1 or 2 detectors (2 or 4 photomultiplier tubes) the detectors are arranged as shown in the following figures. These and subsequent figures illustrate the situation for 96-sample formats.

(A1)	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	
B1	B2	B3	B4	B5	B6	B7	B8	B9	B1Ø	B11	B12	
C1	C2	C3	C4	C5	C6	C7	C 8	C9	C1Ø	C11	C12	Γ
D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	
E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	
F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	
G1	G2	G3	G 4	G5	G6	67	G8	G9	G1Ø	G11	G12	
H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	

One detector model, cassette in first position

(A1)	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	
B1	B2	B 3	B4	B5	B6	B7	B8	B9	B10	B11	B12	
C1	C2	C3	C4	C5	C6	C7	C 8	C9	C10	C11	C12	Two detector
D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	model, cassette in
E	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	first position
F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	
G1	G2	G3	G4	G5	G6	67	G8	G9	G10	611	612	
H1	H2	HЗ	H4	H5	H6	H7	H8	H9	H10	H11	H12	

Six detector model

This model of MicroBeta² has twelve photomultiplier tubes arranged in six pairs. The detectors form a 3 x 2 array, see the figure below.

(A1)	A2	A3	A4(AS)	A6	A7	A8	(A9)	A10	A11	A12	
B1	B2	B3	B4	B 5	B6	B7	B8	B9	B10	B11	B12	
C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	
D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	
E	E2	E3	E4(E	E6	E7	E8	E9	E10	E11	E12	S
F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	n
G1	G2	G 3	G4	G5	G6	67	G8	G9	G1Ø	G11	612	fi
H1	H2	HЗ	H4	H5	H6	H7	H8	H9	H10	H11	H12	

Six detector model, cassette in first position

Six samples are counted simultaneously, which reduces counting time to about one sixth of that for a single detector instrument.

When a cassette is being counted, the transportation system moves it between the detector blocks. The first detector then counts position A1, the second A5 (A3 for 24-well plates), the third A9 (A5), the fourth E1 (C1), etc. (see the figure). At this phase only the results from position A1 are output, because the results must be in the order A1, A2, A3, etc. The cassette moves one position forward and positions A2, A6, A10, E2, E6, and E10 are counted. The results from A2 are output. After counting the whole row the cassette moves to the beginning of the next row (e.g. position B1 in detector 1). The rest of the results from row A are output.

The smallest range of positions is that counted by detector 6. This is therefore the only range that can be counted by all detectors. This affects the positioning of detector normalization and DPM quench correction samples because these routines involve every detector counting the same sample.

Twelve detector model

A twelve detector MicroBeta² has twenty-four photomultiplier tubes arranged in twelve pairs. The detectors form a 4 x 3 array, see the figure below.

(A1) A2 A3 (A4) A5 A6 (A7) A8 A9 (A10) A11 A12	
B1 B2 B3 B4 B5 B6 B7 B8 B9 B10 B11 B12	
C1 C2 C3 C4 C5 C6 C7 C8 C9 C10 C11 C12	Twelve detector
(D1) D2 D3 (D4) D5 D6 (D7) D8 D9 (D10) D11 D12	model, cassette in
E1 E2 E3 E4 E5 E6 E7 E8 E9 E10 E11 E12	first position
F1 F2 F3 F4 F5 F6 F7 F8 F9 F10 F11 F12	
(G1) G2 G3 (G4) G5 G6 (G7) G8 G9 (G10) G11 G12	
H1 H2 H3 H4 H5 H6 H7 H8 H9 H10 H11 H12	

Twelve samples are counted simultaneously, which reduces counting time to about one twelfth of that for a single detector instrument. There are nine readings per plate.

When a cassette is being counted, the transportation system moves it between the detector blocks. Assuming the cassette is for a 96-sample format, the first detector counts position A1, the second A4 etc. At this phase only the results from position A1 are output, because the results must be in the order A1, A2, A3, etc. The cassette moves one position forward and positions A2, A5 etc. are counted. The results from A2 are output. After counting the whole row the cassette moves to the beginning of the next row (e.g. position B1 in detector 1). The rest of the results from row A are output.

A limited range of positions (10 to 12 in row G) can be counted by all detectors. This affects the positioning of CPM detector normalization and DPM quench correction samples because these routines involve every detector counting the same sample.

Protocols

A protocol is a set of parameters for controlling the counting of samples. There are three types:

- Detector normalization protocol
- Quench correction protocol
- MicroBeta² counting protocol

A detector normalization protocol contains parameters used when performing detector normalization and a quench correction protocol contains parameters also for quench correction (see following section on *CPM or DPM counting*). A MicroBeta² counting protocol is used when counting unknown samples. The maximum number of protocols for each type is 100 (0 to 99). Number 0 is the default protocol, which is used if no protocol number is specified. Additional to the three types of protocol listed here, the IPA protocol is intended for monitoring instrument performance and is identified by the protocol number 99.

IPA test normalization

IPA means Instrument Performance Assessment and the IPA test normalization should be run from time to time to make sure that the instrument produces consistent results. An IPA test normalization is similar to a normal detector normalization, but the results are used differently. Data obtained in an IPA test normalization is not used in assay measurements, but is tested against preset limits and then stored so that it can later be compared with other test normalizations using the same nuclide. This comparison is done by presenting the values of some measured parameters as a function of time, so that any systematic trends or large random deviations can be seen easily.

See also the section on *Routine maintenance* which starts on page 39.

Detector normalization and quench correction

CPM counting is used when sample preparation is expected to yield samples with close to constant counting efficiency, i.e. no variation in quench level. This means that the results of the samples in an assay can be compared with each other and used in further data analysis assuming they are all measured by the same detector or by different detectors with known relative efficiencies. In order to determine the relative efficiencies of the detectors in multi-detector versions of MicroBeta² a process called detector normalization is required.

A detector normalization protocol for radionuclide labels as well as glowtype luminescence labels involves the optional measurement of a background plate followed by detection of one or two standards in each detector.

When flash type luminescence labels are used, 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 term quenching is used to describe the situation in which properties of the sample cause variation in counting efficiency. In such a situation, CPM counting can no longer give accurate results, and quench correction must be performed.

The quench correction process involves the use of quench curves obtained by first counting DPM standards with detector 1 and then counting two detector normalization standards with every detector. The curve from detector 1 is recalculated for the other detectors depending on the detector efficiencies.

The use of six DPM standards is adequate for a quench series, although up to 12 can be used with MicroBeta². Each standard should contain the same amount of radioactivity but the amount of quenching agent in the cocktail

should increase incrementally so that the first standard has no quenching agent and the last the greatest amount. The range of the quench standards should be such that it covers the expected quench range of the unknown samples and also that it allows the quench curve to be well defined by the curve fitting program.

MicroBeta² has no external standard so the spectrum quench parameter for the isotope, SQP(I), or the asymmetric quench parameter for the isotope, AQP(I), is used as the index of quench. AQP(I) is used when the Paralux method (see page 20) is used for quench correction in scintillation proximity assay (SPA) assays.

See the MicroBeta² User Manual for more information and instructions on detector normalization and quench correction procedures.

The above explanation implies that single label counting is being performed. DPM results may also be obtained by employing Easy DPM and with dual label counting.

Easy DPM

Easy DPM is a special case of single label DPM counting. 2 active samples, one with quench, one without quench are measured. Based on the results, the software produces a quench curve with these 2 samples and corrects the unknown sample according to the information obtained during easy DPM measurement.

Note! Easy DPM is not available with ParaLux count mode.

Dual label DPM

Dual label counting is similar to single label counting except that two plates are used, one for each nuclide.

See also *Chapter 4*. *Calculation methods* (starting on page 43) for formulae used in DPM counting.

Liquid scintillation counting

OptiPhase[™] SuperMix cocktail

In micro-volume counting very small volumes of sample and cocktail have to be mixed. Traditional cocktails are not well suited for this. A cocktail specifically produced for micro-volume counting is PerkinElmer's OptiPhase SuperMix. Aqueous samples can be mixed without vigorous shaking, and sample loading can be greater than 1 to 1, see the table below. The values are in ml per 10 ml of OptiPhase SuperMix cocktail at different temperatures.

Solute	15°C	20°C	30°C
De-ionized water	7.50	25.00	24.50
0.01M PSB	18.50	19.50	16.50
40% sucrose	13.00	10.50	10.00
1.0M sodium chloride	11.00	9.50	7.50
0.5M sodium chloride	15.50	13.00	10.50
0.1M sodium hydroxide	17.00	17.00	17.00
0.05M Tris HCL	19.50	20.00	19.00
0.1M hydrochloric acid	16.50	15.50	14.00
0.25M ammonium sulfate	14.00	13.50	11.50
0.1M ammonium sulfate	16.00	14.50	13.00
Krebs-Ringer original buffer	16.75	16.75	15.50

The solvent used, di-isopropylnaphthalene (DIN), is in all OptiPhase HiSafe cocktails including OptiPhase SuperMix. DIN is not aggressive, so it does not attack the majority of commonly used plastics (e.g. polyethylene, polypropylene, polycarbonate, polyester and PVC) unlike other LSC solvents. This makes it ideal for use with microtitration plates.

Safety aspects

The toxicological and physico-chemical properties of DIN have been extensively studied and as a result it is classified as a non-hazardous substance. In accordance with international transportation regulations, DIN need not be labeled as flammable or hazardous because its flash point exceeds 65°C (it is 148°C). A biodegradability study of OptiPhase HiSafe II concluded that the compound was readily biodegradable as tested by the ISO 7827-1984 (E) method.

OptiPhase SuperMix, belonging to the OptiPhase HiSafe family, shares these same features which thus greatly simplifies the requirements involved in its transportation and disposal as well as showing it to be both safe to use and environmentally friendly. In many countries it can be disposed of via the drains because of its biodegradability. It is also possible to use a removable adhesive seal for the microtitration plates so that liquid and solid waste can, if necessary, be separated and thus disposal costs reduced. However, it is essential to consult with your Radiological Safety officer and Institute Administrator before entering into any particular course of action.

Filling wells

In the case of the flexible MicroBeta² 96-well plate the maximum total volume of liquid should not be much above 200 μ L; rigid plates have a capacity of 350 μ L. Splashes of scintillant on the plate should be removed with a tissue prior to applying the adhesive seal to prevent the contamination of the detectors.

Caution! Do not overfill wells and take care to remove any splashes of scintillant from the top or sides of the plate.

Chromium release

Specific cytotoxicity can be measured with microvolume samples by counting the supernatant from the chromium release. Add 25 μ L of supernatant to 175 μ L of OptiPhase SuperMix cocktail. The crosstalk between wells is of the order of 0.06% when using the printed microtitration plates. Use a counting window setting of 5 - 170.

Microvolume LSC and chromatography

A chromatography eluent gradient with varying ionic strength can be collected in 96-well plates for evaluation in MicroBeta². The different aliquots will have different levels of quenching and hence different SQP(I) values. The SQP(I) value is then used to obtain the efficiency and hence the DPM for the sample.

In order to automate dispensing to a 96-well plate you can use a device such as the PerkinElmer FlexDrop liquid dispenser.

Scintillation Proximity Assay (SPA)

Introduction

The scintillation proximity assay developed by Amersham (nowadays part of GE Healthcare) is an application for which MicroBeta² with the ParaLux counting feature (see below) is very well suited.

In SPA, fluomicrospheres coated with receptors or acceptor molecules are used for binding the ligand to be assayed. These fluomicrospheres also constitute the scintillation medium. Low energy electrons e.g. tritium beta particles or ¹²⁵I Auger electrons, from the radioactively labeled bound ligand cause light emission from the fluomicrospheres. Labeled, unbound ligand does not cause light emission because of the dilute concentrations used in bioassays where the distances between the unbound ligand and the fluomicrospheres is greater than the maximum range of the low energy electrons; they are absorbed in the aqueous medium. Consequently there is no need to separate the unbound ligand. The whole assay is performed in the microtitration well and the result counted in a beta scintillation counter such as MicroBeta².

Color quenching and SPA

If the SPA involves colored solutions then there may be a need to perform color quench correction. See the MicroBeta² User Manual for more information about the quench correction procedure.

Load the quench correction plate into a cassette with a QCOR label in the FUNC position and a suitable quench correction protocol number and load this quench correction cassette into MicroBeta².

Edit the quench correction protocol and set the quench correction parameters shown overleaf. In particular, select SPA as the assay type. This automatically selects ParaLux counting. Run the quench correction. The results will be saved under the quench correction number marked on the cassette.

When you come to run the actual SPA, set the counting protocol for DPM and give the quench correction protocol number selected above. Other parameters should be set according to the instructions in your SPA kit insert.

ParaLux counting

What is ParaLux counting?

ParaLux is a quench correction method for scintillation proximity assay (SPA). In an SPA assay the scintillating beads settle on the bottom of a well and the scintillation light has to travel a longer distance through the sample solution in order to reach the upper photomultiplier tube. The light reaching the lower PMT has to travel a shorter distance and it does not have to pass through much sample solution. This causes a scintillation light detection asymmetry because events may be detected in the lower tube without their being detected in the upper tube because of quenching. This asymmetry is most pronounced with heavily color-quenched samples.

In the normal counting mode, each pair of PMTs works in coincidence, which means that both must detect the scintillation event for it to be counted. However, in the case described above, there are true scintillation events from the sample which are detected by the lower PMT but which are not detected by the upper tube due to sample absorption and are thus rejected by the coincidence system. These events produce non-coincidence counts.

In ParaLux counting mode, both the coincidence events and the noncoincidence events in the lower tube are counted. In ParaLux quench correction method a quench parameter called AQP(I) (Asymmetric Quench Parameter) is used. This parameter is derived from the difference between the coincidence counts and the counts observed in the bottom PM tube. In quench correction it is used to establish a relation between quench and efficiency.

ParaLux counting modes

ParaLux counting is set in the protocol at the same time as the assay type is selected. It may not be selected if the specified assay type is Normal or Top read, being enabled only when the assay type is SPA.

ParaLux counting, if enabled, may be set to two modes or "none". The two modes are Low background mode (default) and High efficiency mode. The difference between them is as follows.

In the Low background mode, the count rate is calculated from just the coincidence events.

In the High efficiency mode the count rate is calculated from the coincidence events plus the events not in coincidence that are detected in the lower tube. However these latter events are only registered for pulses that

come in channels exceeding the discrimination channel set for the MCA. The default setting for the discrimination channel level is 150. This will normally be a good setting to use, however you may also modify it. If you elevate the level the background will decrease, though it should not be raised too high since the benefit of high efficiency counting will decrease.

Both modes use AQP(I) as the quench parameter, and both modes give improved counting performance because of the use of the Asymmetric quench parameter. Of the two modes, the High efficiency mode gives a higher efficiency but also a higher background.

Radioactive half-life

The half-life of a radioactive nuclide is the time in which half of the radioactive nuclei have decayed, i.e. the activity is halved. The decrease in activity is exponential and can be calculated using the formula:

 $A(1) = A(0)x2^{(-t/T_{half})}$

Where: A(1) = the activity at time t(1), A(0) = the activity at time t(0) and t=t(1) - t(0). That f = the half-life of the nuclide.

Each radioactive nuclide has its specific half-life. If the half-life of a nuclide to be counted is short, and if the counting time of a batch of samples is relatively long, the last samples will have decayed more than the first ones so the results between earlier and later samples cannot be directly compared with each other. As an example: the half-life of the ³²P nuclide is 14.3 days, and if counting takes 24 hours, the last samples will give about 5 % lower activity than they would have done if counted at the same time as the first sample.

Half-life correction

The half-life correction is calculated if the appropriate box is checked under the corrections tab during protocol setting.. This correction is recommended if the half-life of the nuclide used is short and/or the total counting time is long.

Nuclide	Half-life	Half-life hours
³ H	12.3 years	107500
^{14}C	5760 years	-
^{32}P	14.3 days	343
³⁵ S	87.2 days	2093
⁵¹ Cr	27.8 days	667
¹²⁵ I	60.0 days	1440

If you use half-life correction then if a batch is recounted after, say, one week, the two sets of results are directly comparable with each other.

Note! When working with half-life correction, always check that the computer date and time settings are correct.

Energy windows

Radioactive nuclides disintegrate spontaneously. The disintegration results in emissions of alpha, beta or gamma radiation. The radiation type and the energy emitted is specific for each nuclide. In the case of beta radiation the emitted beta particles have a wide range of energies, the energy spectrum forming a continuous distribution.

Beta-radiation energy ranging from 0 to 2000 keV can be measured in MicroBeta² (the most common beta emitters have their energies between these energy values). The energy scale is converted into a logarithmic scale and divided into 1024 channels. The energy spectrum of a beta emitting nuclide falls into a certain segment of the energy scale, called an energy window. A window is a part of the energy scale consisting of many adjacent channels e.g. a window beginning from channel 5 and extending to channel 360 will cover the energy spectrum of tritium in MicroBeta².

Window setting

The MicroBeta² LSC has built in windows for the nuclides, ³H, ¹²⁵I, ¹⁴C, ³⁵S, ³³P, ⁵¹Cr, ³²P, ³²P Cerenkov and for Luminescence counting. The window for Other can be set by the user. The default value is 5-1024. The preset energy windows shown below are used for these nuclides when the nuclide is chosen in the protocol. When measuring dual labeled samples any of these window combinations can be used.

Note! Before counting, MicroBeta² should be normalized for each nuclide used.

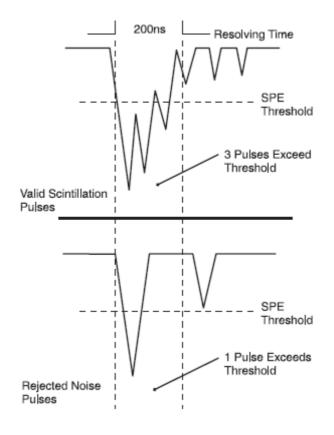
Nuclide	Channel settings
³ H	5 - 360
¹²⁵ I	5 - 530
^{14}C	150 - 650
³⁵ S	150 - 650
³³ P	150 - 650
⁵¹ Cr	5 - 170
32 P	250 - 960
³² P Cerenkov	5 - 1024
Other	5 - 1024
Luminescence	5 - 1024

If only upper or lower photomultiplier tubes are used, then the default window is 150 -1024 for non-luminescence counting. The lower window setting is increased from 5 to 150 to prevent the detection of detector background which typically shows at lower window settings.

TR-LSC counting

With measurements using only the upper photomultiplier tube (PMT), the TR-LSC (time-resolved scintillation counting) method makes it possible to distinguish between true scintillation events and background noise.

A scintillator with a long decay constant will emit photons over a long time, until all of the absorbed energy has been released. Each photon results in a discrete pulse at the PMT. Therefore, each decay event produces a photon packet consisting of a series of pulses. In contrast, PMT noise consists of single pulses. The characteristics of a pulse are determined by observing the PMT output for a period of time after the initial pulse has been detected. If it is followed by one or more additional pulses, the pulse probably results from a true decay event, and it is accepted. If no additional pulses are detected within the resolving time period, the initial pulse was probably background noise, and it is rejected. Recognition of either two or three pulses is sufficient to distinguish valid pulses from background noise for all of the long lifetime scintillators used with MicroBeta².



TR-LSC eliminates the requirement for two PMTs to count each sample. It is particularly appropriate for Top read assays involving opaque bottom sample matrices like Lumaplate and Unifilter plates.

MicroBeta² supports three TR-LSC count modes: High efficiency, Normal and High sensitivity.

High efficiency count mode

This counting mode maximizes counting efficiency for assays that can tolerate relatively high nonspecific signals.

- Certain single-photon and all multiple photon events are accepted as true decays.
- Low-energy, single-photon events are rejected as background by an adjustable lower level discriminator.
- High efficiency count mode can be used to enhance the response for low signal FlashPlate, PVT-SPA and Cytostar-T assays.
- It is also used in Cerenkov counting applications.

Normal count mode

This mode optimizes both counting efficiency and signal-to-noise ratio for assays with low non-specific signal.

- Detected events must have two or more photons to be accepted as a true decay.
- Single-photon events are rejected as background.
- Normal count mode is the recommended mode for most liquid scintillation methods using MicroScint or FlexiScint, and proximity assays using FlashPlate, PVT-SPA beads or Cytostar-T.

High sensitivity count mode

This mode maximizes signal-to-noise ratio for measuring high energy radionuclides and those assays which use long lifetime scintillators.

- Events must have at least three photons to be accepted as true decays.
- All single- and dual-photon events are rejected to reduce backgrounds to very low levels.
- High sensitivity count mode is used primarily for LumaPlate and SPA applications using yttrium silicate beads.

Top read assays

Top read is the assay type selection that is needed for assays carried out on opaque bottom microplates such as FlashPlate, Lumaplate or on Unifilter plates. The signal is detected by the upper detector only.

On selection of the Top read assay type, TR-LSC counting is set as default in order to provide effective background reduction.

P-32 Dot blot quantification

Assays utilizing the ³²P label and 96-format filtermats, e.g. dot blot hybridizations can be detected with MicroBeta² using the ³²P cassette (1450-118). Dried sample membranes can be counted directly in the cassette, while wet membranes need to be counted in a sample bag, to avoid any contamination. The counting method does not include any scintillator addition to the actual filter and thus allows re-probing of the samples. When ³²P samples are measured with the 1450-118 cassette the obtained results are quantitative. The ³²P counting efficiency is around 70% and background less than 10 CPM. Crosstalk values less than 0.05 % are achieved.

The cassette consists of a cassette body and a lid. In the cassette body there is a steel plate, which is the same size as the filtermat. The filtermat is aligned on the steel plate according to the edges and the lid is clicked on the filtermat, so that the small hole is in the upper left corner, the A1-corner.

To count the ³²P cassette in MicroBeta², choose other isotope, upper PMT usage and window 350-1024 in your detector normalization or counting protocol. No crosstalk correction is needed. If you use Windows workstation you can use the parameter ³²P cassette 8 by 12 for ³²P membranes or you can set up a new parameter with the settings previously mentioned.

Luminescence counting

Luminescence assays come in two main forms; those making use of luminescence that has a short signal (flash luminescence) and those making use of that which emits a long stable signal (glow luminescence). Both can be measured with MicroBeta², however the short emission assays require reagent addition. This means that for flash luminescence you must use 2460 MicroBeta² LumiJET, which has an injector module or modules installed.

Glow luminescence assays can be measured with either MicroBeta² version without using injection.

Glow luminescence assays

Luminescence assays carried out on a solid support e.g. nylon membrane, glass fibre or coated plate, and in solution can be directly quantified.

Traditional microtitration plates are totally opaque (black or white). These plates are counted with the upper tube and a 1450-105 cassette is used. Filters are counted with the upper or lower tube and a 1450-104 (96-well plate format) or 1450-116 (24-well plate format) filter cassette is used.

The luminescence units used are CCPS, corrected counts per second. Before counting the actual samples, the detector and hence the CCPS-values should be normalized with a luminescence solution as similar to the actual samples as possible.

Flash luminescence with MicroBeta² LumiJET

In addition to the features described above, MicroBeta² LumiJET makes it possible to measure flash luminescence, dual luminescence and do kinetic studies because it allows reagent addition and mixing by dispensing.

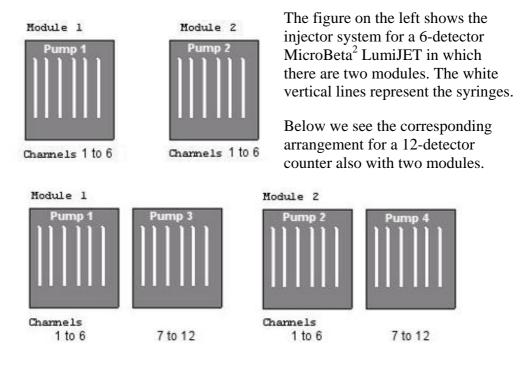
An instrument can have up to two injector modules, each with as many channels as there are detectors in the instrument i.e. 1, 2, 6 or 12. The use of two injectors for the same well means that dual luminescence assays can be performed.

Each module and channel can be switched on or off independently. This is done by modifying the plate map. If samples in a certain area are not marked for measurement, injectors on those areas will not dispense liquid either.

If reagent injection is selected in any particular counting protocol, the mask adapter (injector assembly) is driven into position above the microplate. The injector tips, which are embedded in the mask adapter are automatically positioned over the wells to allow injection under optimum conditions. The light resulting from the reaction passes through the fibre optic coupling in the injector assembly to the upper PMT.

The mask adapter can also be used without injection simply for masking. It reduces the signal to the upper detector by a factor of ten, which can be helpful in cases of luminescence with very high signal.

For 1-6 detector instruments an injector module is a pump and feeding tube system with 1, 2 or 6 or syringes. For 12-detector instruments one injector module includes 2 pumps each with 6 syringes. Each syringe with its associated tubing constitutes one channel.



Caution! Do not allow the pumps to run continuously without liquid. This can damage the sealing surfaces.

Plastics used in injector modules

Plastic materials used in injector modules comprise: Telfon[®] (PTFE, TFE, FEP) in tubing, valve plugs and seals Kel F[®] in valve bodies Polypropylene in fittings for tubing

Caution! Acetyl chloride, acetylene, arochlor 1248 and aromatic hydrocarbons will have a severe effect on polypropylene, and these compounds are not recommended for ANY use.

For more information on chemical compatibility please refer to PerkinElmer or to Tecan Systems, Inc, the manufacturer of the Cavro[®] XLP 6000 and Cavro XMP 6000 Modular Syringe Pumps, used in MicroBeta².

Injector setup, and steps needed after use

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

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 in the Ready state.

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. See the **Move Out** command in the Injector Setup dialogue.

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

Injector Setup	
Injector Assembly Move In Move Out Injector Module	Close
Module 1 💌 Last Action	κ.
Preparation	Maintenance
	Recover
Prime	Wash 1 mL
Tip	
Dispense 10 µL	
Status: Ready	

The software checks if the injector assembly is in or out and enables the **Injector Assembly** buttons accordingly.

The Injector Setup dialogue 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 settings needed before counting are described in more detail in the Help.

After use, it is important to remember the following:

- Unused reagent should be recovered and saved by clicking the Recover button.
- Tubing must be washed with 50% ethanol solution and rinsed with distilled water, although the procedure followed might depend on the reagent used. An alternative practice should be followed if recommended by the reagent manufacturer. As the final step, the tubing should be emptied.
- The dispensing head must be kept dry and the fibre optic clear. Clean with a moist cloth. Always use distilled water.

Dual screen mode

When an assay involves two dispensing stages, dual screen mode allows the plate the plates measured in two sequential steps. In other words, the second dispensing stage is started only after a first measurement has been performed for all wells.

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.

Dual screen mode is especially intended for Aequorin applications where antagonists are also screened. First cell suspension is dispensed to the wells and potential agonist signal is measured. The agonist solution is then dispensed and this is followed by a measurement for potential reduction in the control signal caused by a possible antagonist effect of the compound.

Use of dispensers for maintaining cell-based samples in suspension

When working with cell-based samples, dispensers can be used to prevent cells from settling to the bottom of the vial or aspiration tubing. When the **Keep cells in suspension** option is selected using the MicroBeta² Workstation software pump module 1 will aspirate cell solution into the syringes and push it back to the reservoir. The resulting liquid flow mixes the cell suspension. The mixing step is performed once before the assay, every time after a dispensing step and once every minute when the assay is

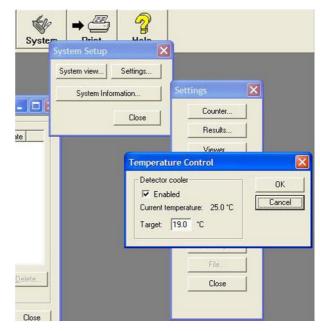
in pause mode or waiting for a new plate. This mode requires a minimum volume of cell suspension solution, which is 500 μ L times the number of syringes in the module 1. In 12 detector unit this is 12x500 μ L = 6 mL plus some extra to maintain the tubing in solution and prevent aspiration of air.

Luminescence option

When the Luminescence option is installed it allows the temperature of the upper detectors to be reduced and stabilized. This lowers the luminescence counting background because the cooling decreases the photomultiplier tube thermal background. E.g. changing the detector temperature from 27° C to 20° C decreases the thermal background by about 60 %. It is possible to measure luminescence without this luminescence option, too. In such a case background will be slightly higher, and reduced S:N ratios will be obtained in the assays.

The luminescence option can be used to cool the upper detectors to as much as 5 $^{\circ}$ C (12 detector models) or 7 $^{\circ}$ C (1-6 detector models) below room temperature.

The detector temperature can be selected by clicking the **System** button on the toolbar to obtain the **System Setup** dialogue. Click **Settings** and **Counter**. See that the detector cooler is enabled and specify your desired target temperature.



Monitors and flags

DPM out-of-range monitor

This monitor evaluates quench levels of samples in DPM counting and compares these with corresponding quench levels in the quench correction. This is done by measuring SQP(I) for a sample. If it is not within the quench correction range the monitor gives a DPM out of range message.

The DPM out-of-range monitor is included in the short and long outputs in DPM counting. It is no longer in use if it has been removed from the outputs.

Termination flag

Counting is normally terminated when the specified counting time is reached or when the user stops it, but in some cases it can be interrupted earlier, on the basis of precision, for example. The termination flag is set to

Item name	Heading	Value	Explanation
DPM out of range	DPMM		OK, no out of range
monitor			detected
		OUT	Out of DPM range
Termination flag	FLAG	•	Normal termination time
			reached
		PREC	Precision reached
		INT	Counting interrupted by
			user

'PREC' if the precision value has been reached. The counting stops if the precision has been reached in all detectors.

The flag is set to 'INT' if the user interrupts the counting by selecting Off, Next position or Next protocol.

Total count rate

A total count rate register is provided for each of the 7 standard nuclides and one register for the user definable nuclide (called Other). At the end of each assay a total accumulated count rate or activity for that assay is added to the end of the result file

Robotic loading interface

The robotic loading interface is a special door/adapter system that brings a cassette outside so that a robotic arm can change the plate. In this case only one shelf is used.

Note! The mask adapter has to be moved manually into the counting chamber before starting counting.

Microcomputer control

MicroBeta² has its own built-in microcomputer with LAN and USB connections. There is an external monitor and keyboard allowing operation using the MicroBeta² Windows Workstation software.

Chapter 3 Information about user instructions and warnings

Information about user instructions and warnings

User instructions

There are several forms of user instructions:

User manual

This is a separate manual from this instrument manual. It gives information on how to use the instrument, with special emphasis on the $MicroBeta^2$ Workstation software.

Quick start guide

This is separate short manual that gives the most basic information needed for a routine user to operate the system.

On-line help

This is supplied with the software and can be accessed from the **Help** menu, or by pressing F1 on the keyboard.

On-line help consists of short information about all features of the operation that concern the normal user (service information is not provided).

Installation and maintenance

Installation may only be performed by PerkinElmer-trained and authorized personnel.

Routine maintenance that should be performed by the user is described in a separate chapter of this instrument manual (see page 39). Any other maintenance than that described there should be performed by a service PerkinElmer-trained and authorized specialist.

Printed warnings

Below we describe the printed warnings that you will see on or associated with the instrument.

Regarding connection of the instrument to the mains

Note! The instrument must be connected to a mains supply having a protective earth.

On the back of the instrument Warning! Disconnect supply before servicing

Safety symbols used



This symbol warns of the danger of mechanical injury, and is associated on the instrument with the pumps and the robot loading door.

Power ON

O Power OFF

Additional hazard warnings

The following hazard warnings should be carefully observed. For additional important warnings please see also page 1.

Mechanical hazard

Automatically moving parts may cause injuries (crushing, piercing). Although a door lock prevents access to the sample cassette changer mechanism while it is operating, care should be taken with accessible moving parts.

Warning! Keep fingers out of the syringe slot while the pump is running. Failure to do so can cause injury.

Bio-hazard

Your laboratory is responsible for ascertaining the hazard status of sample materials and for implementing appropriate rules for handling and disposal of samples.

Warning! All samples containing human or animal tissue, blood, other body fluids or their derivatives should be treated as bio-hazardous, and handled and disposed of accordingly.

Contact addresses

World Headquarters

PerkinElmer Life and Analytical Sciences, 940 Winter Street, Waltham, Massachusetts 02451, USA. Tel. (800) 551-2121

European Headquarters

PerkinElmer Life and Analytical Sciences, Imperiastraat 8, B-1930 Zaventem, Belgium. Tel. 32 2 717 7911

Manufacturer

PerkinElmer Life and Analytical Sciences, Wallac Oy, P.O. Box 10, FIN-20101 Turku, Finland. Tel: 358-2-2678111 Fax: 358-2-2678 357 Email: info@perkinelmer.com Website: www.perkinelmer.com

Service

Please contact your local PerkinElmer representative.

Chapter 4 Routine maintenance

Routine maintenance

Cleaning the instrument

The cassette rack should be kept clean. The cassettes should be kept clean in order to prevent dirt from coming between the detector blocks, to minimize the background and maximize the light collection efficiency.

In MicroBeta² LumiJET the optical fibers of the mask adapter (injector assembly) must be kept clean. If necessary, wipe them with a moist cloth.

Tubing should be washed with water using the Wash command.

Note! For additional information on cleaning tubing it may be advisable to check manufacturer instructions for the reagents you use. In addition, refer back to the Luminescence description of this manual for information on plastics used in the manufacture of injector modules (page 27) and steps to be taken after use of injectors (page 28).

ID label

Check the labels on the ID support plates. Those in bad condition should be replaced by new ones to guarantee correct reading of the labels. When fixing ID labels on the support plate, ensure that the area where the label is to be fixed is clean, e.g. that there is no perspiration from your fingers on it.

Power supply fan

Check that the cooling fan in the power supply unit is working.

Checking the instrument

To check the instrument, it is recommended that at least once a month you run counting protocol number 99, the IPA test normalization protocol (see page 16). Use this protocol to count the detector normalization sample plate supplied with the instrument. The background, ³H and ¹⁴C samples are counted with all detectors so background level, CPM values and SQP(I) values can easily be compared.

Recommended limits:

³ H		¹⁴ C	
Eff%	Bkg	Eff%	Bkg
>45%	max 50 cpm	>91	max 60 cpm

If the values obtained by running the IPA test normalization protocol are outside the recommended limits, please contact your service support person.

Chapter 5 Calculation methods

Calculation methods

Count rate

Count rate i in counts per minute (headings **CPM**, **CPM1**, **CPM2**, **CPM3**) is calculated using the equation:

i =60*c/t

(1)

where c is counts in the counting window and t is dead time corrected counting time (heading **CTIME**) in seconds.

In ParaLux high efficiency mode:

 $CPM1 = CPM_C + CPM_NC$

where CPM_C is normal coincidence count rate and CPM_NC is noncoincidence count rate between discriminator channel and 1024.

Count rate α-sigma percentage error (headings CPM%, CPM1%, CPM2%, CPM3%) is calculated using the equation:

 $\alpha * \delta i / i = \alpha * 100 / \sqrt{c}$

(2)

Above and in what follows α can be either 1.0 or 2.0 .

Detector normalization

Detector efficiency normalization

Detector efficiency detector normalization corrects for differences in detector responses. Efficiencies and efficiency errors of each detector are calculated and stored for count rate correction. Background corrected count rate r is calculated using the equation

 $\mathbf{r} = \mathbf{i} - \mathbf{b} \tag{3}$

where i is from equation (1) and b is the detector normalization background count rate for the same detector and counting window. ParaLux high efficiency backgrounds are subtracted first.

Efficiencies e(mn) are calculated using the equation

e(mn) = r(mn) / j(4)

where r(mn) is nuclide n background and half-life corrected count rate in window m and j is the given detector normalization activity or the maximum background corrected count rate of nuclide n in window 1 (single label) or in window 5-1024 (dual label) or the average count rate (luminescence).

Efficiency percentage error is calculated using the equation

 $\delta e(mn)/e(mn) = \sqrt{\{[\delta i(mn)/r(mn)]^2 + [\delta b(mn)/r(mn)]^2 + [\delta j/j]^2\}}$ (5)

If j is the given activity then $\delta j=0$.

Corrected count rate

Corrected count rate (headings **CCPM**, **CCPM1**, **CCPM2**) is calculated in three steps in the following order:

- Background correction
- Detector efficiency correction or crosstalk correction
- Half-life correction

The first two corrections also affect the theoretical error (headings CCPM%, CCPM1%, CCPM2%) of the corrected count rate.

Background correction

If background sample is specified and measured or background is given in the protocol then count rate i is corrected for background using equation (3).

r = i - b

ParaLux high efficiency backgrounds are subtracted first.

If background sample is measured then the count rate α -sigma error becomes:

$$\alpha^* \delta r / r = \alpha \sqrt{\left[(\delta i)^2 + (\delta b)^2 \right] / r}$$
(6)

Detector efficiency correction

In single label counting detector efficiency correction for count rate is calculated using the equation

 $\mathbf{R} = \mathbf{r} / \mathbf{e} \tag{7}$

where r is from equation (3) and e = e(11) from equation (4) for the same detector as in detector normalization.

Efficiency correction affects also the corrected count rate α -sigma error,

$$\alpha^* \delta R / R = \alpha \sqrt{[(\delta r/r)^2 + (\delta e/e)^2]}$$
(8)

where $\delta e/e = \delta e(11)/e(11)$ from equation (5) and $\delta r/r$ is from equation (6).

In dual label counting the efficiency correction has the form

(9)

$$R(m) = [e(nn)*r(m) - e(mn)*r(n)] / d$$

where

$$d = e(mm)*e(nn) - e(nm)*e(mn)$$
 (10)

and e(mn) stands for nuclide n efficiency in window m.

Corrected count rate α -sigma percentage error in the dual label case is calculated using the equation

$$\alpha^* \,\delta R(m)/R(m) = \alpha^* 100 \sqrt{[[r(m)^2 * \delta e(nm)^2 + e(nm)^2 * \delta r(m)^2 + r(n)^2 * \delta e(mm)^2 + e(mm)^2 * \delta r(n)^2]/p(m)^2 + Q/d^2]}$$
(11)

where r(m) is from equation (3), $\delta r(m)$ is from equation (6), d is from equation (10),

$$p(m) = e(nn) * r(m) - e(nm) * r(n)$$
(12)

and

$$Q = e(mn)^{2} * \delta e(nm)^{2} + e(nm)^{2} * \delta e(mn)^{2} + e(nm)^{2} * \delta e(mm)^{2} + e(mm)^{2} * \delta e(nm)^{2}$$
(13)

Half-life correction

Half-life corrected count rate I is calculated using the equation

$$I_{\tau} = [[(t/\tau)*ln(2)]/\{exp[-ln(2)*(T-t)/\tau]-exp[-ln(2)*(T/\tau)]\}]*R$$
(14)

where t is counting time, T is elapsed time from zero time to the end of counting, τ is the half-life of the nuclide and R is the background and detector efficiency corrected count rate. Both single and dual label corrections have the same form. Half-life correction has no effect on the corrected count rate errors.

Spectrum indexes

Isotope spectrum end point

Isotope spectrum end point (heading **ISEP**) is a channel at the end of the spectrum.

Spectrum quench parameter

Spectrum quench parameter of the isotope spectrum (heading **SQP(I**)) is the spectrum mean pulse height and is calculated using the equation

$$\mathbf{q} = \sum [\mathbf{i} * \mathbf{c}(\mathbf{i})] / \sum \mathbf{c}(\mathbf{i}) \tag{15}$$

where i = window 1 low ... Max (window 1 high, window 2 high, window 3 high) is the channel number and c(i) is the number of counts in channel i. The SQP(I) counting window is truncated so that a 10 counts range is removed from both ends.

The α - sigma percentage error of SQP(I) is given by:

$$\alpha^* \delta q/q = \alpha^* 100 \sqrt{\{\sum [i^2 c(i)] / \sum c(i) - q^2\} / \sum c(i)) / q}$$
(16)

Asymmetric quench parameter

Asymmetric quench parameter (heading **AQP(I)**) is a measure of the isotope spectrum for an asymmetric sample and varies as the quench changes. It establishes a relation between quench and efficiency.

Quench correction

Activity quench correction

Quench correction corrects for differences in sample quench levels and in detector responses.

All standard samples are counted with detector 1. Only the unquenched standard N1 and the fully quenched standard N2 are counted with all detectors. The SQP(I)s or AQP(I)s, efficiencies and weights of standard samples are calculated and stored for activity calculations.

The efficiency in a counting window is

$$\mathbf{E} = \mathbf{r}/\mathbf{a} \tag{17}$$

where r is background and half-life corrected count rate and a is the given activity of the standard sample.

Quench correction weight is given by

$$w = \sqrt{[(k^* \delta q)^2 + (\delta r/a)^2]}$$
(18)

where k is the slope of the standard curve at q, δq is from equation (16) and δr is from equation (6).

Activity

Using activity quench correction

Sample activity in disintegrations per minute (headings DPM and DPM1) is

$$A = r/E \tag{19}$$

where r is background corrected count rate (3) and E is counting efficiency (17) at SQP(I) or AQP(I). Activity is half-life corrected using equation (14).

Efficiency E is obtained from the quench correction efficiency curve of detector 1 using the measured SQP(I) or AQP(I) of detector i.

The measured SQP(I) or AQP(I), q(i), is first corrected to correspond to detector 1,

$$q = q(1,N2) + [\{q(1,N1)-q(1,N2)\}/\{q(i,N1)-q(i,N2)\}]*[q(i)-q(i,N2)]$$
(20)

where the first index stands for the detector number and the second index stands for the standard sample type.

If the measured SQP(I) or AQP(I) is not between the quench correction SQP(I) or AQP(I) limits, then linear extrapolation with the two first or last points is used (or spline with the two last points if smoothing spline is selected). If the DPM out-of-range monitor (heading **DPMM**) is selected then OUT is printed to warn the user.

The interpolated efficiency is corrected to correspond to detector i using the equation

$$E = E(i,N2) + [\{E(i,N1)-E(i,N2)\}/\{E(1,N1)-E(1,N2)\}]*[E(1)-E(1,N2)]$$
(21)

Activity α -sigma percentage error (headings **DPM%** and **DPM1%**) is given by

$$\alpha^* \delta A / A = \alpha \sqrt{[(\delta r/r)^2 + (\delta E/E)^2]}$$
(22)

where $\delta r/r$ is count rate error (6) and efficiency error comes from the equation

$$\delta \mathbf{E} = \sqrt{[(\mathbf{k}^* \delta \mathbf{q})^2 + \mathbf{w}^2]}.$$
(23)

Here k is the slope of the standard curve and w is the quench correction weight at q.

Chapter 6 Specifications

Specifications

This section contains information about the safety standards and lists MicroBeta² technical information.

Safety standards

Electrical safety requirements

The design of the instruments is based on the following electrical safety requirements:

EN 61010-1 Safety requirements for electrical equipment for measurement, control, and laboratory use

EN 61326-1 Electrical equipment for measurement, control and laboratory use – EMC requirements

Certification

• IEC-CB, CE and NRTL-TUV Rheinland of North America

The instrument fulfills the requirements of:

- IEC 61010-1:2001 (Second Edition)
- CAN/CSA-C22.2 61010-1:2004
- UL 61010-1:2004 R7.05

Operational safety

The safety specifications are met under the following environmental conditions in addition or in excess to those listed below under **Power requirements** and **Environmental requirements**.

Temperature: $+5^{\circ}C$ to $+40^{\circ}C$

Relative humidity: Maximum 80% at 31°C decreasing linearly to 50% at 40°C

Mains supply fluctuations: ±10%

Installation category (overvoltage category): II according to IEC 664-1 (see first note below)

Pollution degree: 2 according to IEC 664-1 (see second note below)

Note! Installation category (overvoltage category) defines the level of transient overvoltage that the instrument is designed to withstand safely. It depends on the nature of the electricity supply and its overvoltage protection means. For example in CAT II, which is the category used for instruments in installations supplied from supply comparable to public mains, such as hospital and research laboratories and most industrial laboratories, the expected transient overvoltage is 2500 V for a 230 V supply and 1500 V for a 120 V supply.

Note! Pollution degree describes the amount of conductive pollution present in the operating environment. Pollution degree 2 assumes that normally only non-conductive pollution such as dust occurs with the exception of occasional conductivity caused by condensation.

Both of these affect the dimensioning of the electrical insulation within the instrument.

The CE mark conforms to the following EU directives	 2006/95/EC Electromagnetic Compatibility 73/23/EEC (as amended by 93/68/EEC) Low Voltage 98/79/EC In Vitro Diagnostics Medical Devices (IVDMD)
Performance specifications used to verify conformance to the Directives above	 EN 61326 class B Requirements IEC 61010-1:2004 (Second Edition)

Conformance to EU directives

Physical specifications

Dimensions

	2450 MicroBeta ²	2460 MicroBeta ²
		LumiJET
Width	430 mm	700 mm
Height	630 mm	630 mm
Depth	645 mm	645 mm
Depth for robot	795 mm	795 mm
loading models		
Weight	80 kg	85 kg

Power requirements

100 – 240V at 50/60 Hz

250 VA maximum (applies to both 2450 and 2460 instruments)

Environmental requirements

Temperature +15°C to +35°C. Relative humidity: 10% to 80% Altitude up to 2000 m.

Sample capacity

Sample capacity using 96-well sample plates is 16*96 = 1536 samples, using 24-well sample plates 16*24 = 384 samples, using 4 mL vials or Eppendorf tubes 8*24 = 192 samples, and using 384-well sample plates 16*384 = 6144. With 2450 MicroBeta² counters, capacities may be doubled by using 32 shelves instead of 16. Any combination of cassettes either for 96 or 24-well sample plates or 96 or 384-well sample plates is possible.

Features

External power supply

Power failure recovery and protection can be obtained with an external UPS back-up power supply provided. This can be ordered from PerkinElmer Life and Analytical Sciences.

Computer and external communication

The counter's built-in industry standard computer runs under Windows XP SP3. It has USB connections for memory stick, external hard drive and printer.

Ethernet connection for connecting to a LAN is also available. By means of a LAN connection, MicroBeta² can be controlled via an external computer, and results can be sent to LAN-based external devices such as a printer.

Connections/input/output

Serial ASCII interface RS-232
 USB ports at the back of the instrument
 Ethernet 10/100 port

Sample identification system

Each cassette can be provided with a 1450-451 ID Support Plate for 2450-4010 ID Labels. An ID Support Plate has marked areas for ASSAY, PROTOCOL and CASSETTE numbers (0 - 99) and the FUNCTION codes NORM (detector normalization), QCOR (quench correction) and STOP.

There is also an optional Plate ID reader.

Counter electronics

Logarithmic A/D converter energy range 1 - 2000 keV. There is a 1024channel multichannel analyzer for each detector. Built-in dead time correction.

Performance

(100 ns coincidence time)

Background

(at the factory, Turku, Finland):

With empty 1450-514 plate:

<15 CPM in wide ³H window

<17 CPM in wide ¹⁴C window.

Unquenched sample with a volume of 150 microliters high flash point cocktail unpurged in the 1450-401 96-well Sample Plate:

typically 6 CPM in wide ³H window typically 9 CPM in wide ¹⁴C window

Unquenched sample with a volume of 750 microlitres high flash point cocktail unpurged in the 1450-402 24-well Sample Plate:

typically 24 CPM in wide ³H window typically 37 CPM in wide ¹⁴C window

Efficiency

1450-471 Detector normalization standard $^{3}\text{H} > 45$ % $^{14}\text{C} > 90$ %

Unquenched sample with a volume of 150 microlitres high flash point cocktail unpurged in the 1450-401 96-well Sample Plate:

³H: typically 57 % ¹⁴C: typically 94 %

Unquenched sample with a volume of 750 microlitres high flash point cocktail unpurged in the 1450-402 24-well Sample Plate:

³H: typically 61 % ¹⁴C: typically 95 %

Crosstalk

Optical crosstalk due to light piping in the plastic of the sample plates. The values are given for sample plates with black printing inhibiting crosstalk between the sample wells.

Unquenched sample with a volume of 150 microlitres high flash point cocktail unpurged in the 1450-401 96-well Sample Plate:

In wide ³H window: < 0.05 % In wide ¹⁴C window: typically 1.8 % Unquenched sample with a volume of 750 microlitres in the 1450-402 24well Sample Plate:

In wide ³H window < 0.02 %

In wide ¹⁴C window: typically 0.2 %

Crosstalk for luminescence in 384-well plates is app. 0.02 %

Measure luminescence crosstalk for 96-well plates.

Stability

Count variation less than 0.5 % / 24 hours (not including random statistics).

Program Specifications

Counting protocols

100 fully programmable counting protocols. Start of counting manually through keyboard command or automatically by the use of ID labels.

Delay Start, allows for sample incubation prior to start of measurement.

Edit, Copy, Print, Delete, List features for management of protocols.

Password protection against unwanted changes of protocol.

Help function, built-in operating instructions supports the user throughout the program.

Single label counting with or without quench correction.

Dual label counting with or without quench correction.

Crosstalk correction to allow different types of microtitration plate to be used.

Scintillation proximity assay can be done.

Preset parameter for 6 common nuclides.

Count termination by counting time or reached precision (2 sigma error).

Plate counting control, whole plate or only active rows or positions.

Default, Short or Long printer output for quick printout settings.

Fully programmable printout selection. Including statistical analysis of results and user functions.

Fully programmable output to display. Including statistical analysis of results and user functions.

Fully programmable output to PC or network file. Including statistical analysis of results and user functions.

Repeat or Replicates counting.

Cycle counting, repeat counting of the whole assay.

Background correction. CPM/DPM results can be corrected for experimental background either by typed in background value or background samples.

Programmable counting time for background samples.

Half-life correction, corrects the CPM/DPM values for decay of short-lived nuclides. Zero time start of assay or specified date and time. Chemiluminescence correction, corrects the CPM/DPM values for random coincidence contributions.

Statistics Monitor, detects distorting contributions from static electricity, associated with the use of plastic sample carriers such as microtitration plates.

Detector normalization protocols

Up to 100 password protected Detector normalization protocols.

The Detector normalization protocol to be used is selected in the counting protocol. Also available in the protocol are:

- ➢ Half-life correction.
- Chemiluminescence correction.

Quench correction protocols

Up to 100 password protected Quench correction protocols.

Selection of curve fit method; smoothed spline, interpolation spline, linear interpolation or linear regression.

Input of individually adjusted DPM values for the standard samples.

The Quench correction protocol to be used is selected in the counting protocol. Also available in the protocol are:

- Chemiluminescence correction.
- ➤ Half-life correction.

Password protection on pre-installed protocols

Pre-installed counting, detector normalization and quench correction protocols are protected by a password, which is initially set to be *PerkinElmer*.

Counter construction

Sample cassette changer

A z-direction moving cassette rack with shelves for 16 or 32 cassettes. This rack functions as a random access stack, which allows access to any cassette in the rack. The cassette rack is driven by a stepper motor and its movement is controlled by electro-optical sensors. A transportation system moving cassettes in the x-y-direction selects one cassette from the cassette rack and takes it into the counting position and then returns the cassette to the

cassette rack. This transportation system is driven by two stepper motors and is controlled by electro-optical sensors. Only the 16 shelf sample cassette changer may be used with MicroBeta² LumiJET counters.

MicroBeta² LumiJET injector system

One injector module dispensing at a time, allowing reading before dispensing, reading after dispensing and delay time after dispensing.

Plastic materials used in injector modules comprise: Telfon[®] (PTFE, TFE, FEP) in tubing, valve plugs and seals Kel F[®] in valve bodies Polypropylene in fittings for tubing

Caution! Acetyl chloride, acetylene, arochlor 1248 and aromatic hydrocarbons will have a severe effect on polypropylene, and these compounds are not recommended for ANY use.

For more information on chemical compatibility please refer to PerkinElmer or to Tecan Systems, Inc, the manufacturer of the Cavro[®] XLP 6000 and Cavro XMP 6000 Modular Syringe Pumps, used in MicroBeta².

Mask adapter (Injector assembly)

A transportation system for moving the mask adapter in the x-direction from the maintenance tray into the counting chamber under the upper detector block. This transportation system is driven by one stepper motor and is controlled by electro-optical sensors. The mask adapter moves in the zdirection with the upper detector block.

Detector assembly

1, 2, 6 or 12 detectors (each consisting of a pair of 19 mm PM tubes working in coincidence), are mounted in two detector assemblies, the upper detector assembly moving in the z-direction. PM tubes are Hamamatsu R1166 or equivalent. Coincidence resolution time is 100 ns. One to six detector assemblies are provided with circular apertures for 96 and 24-well sample plates. The 12-detector assembly is provided with circular apertures for 96-well sample plates and square apertures for 384-well sample plates. The apertures are automatically changed to correspond to the plate type.

Consumables

Sample Plates

1450-401 96-well Sample Plate (25/box): 96 round-bottomed wells, 8x12 format, made of clear PET-plastic, printed lines between wells, chemically resistant to all HiSafe-cocktails, max. volume 250 μ L/well. Sealed with tape type 1450-461 or 1450-462. Counted in cassette type 1450-101.

1450-402 24-well Sample Plate (25/box): 24 flat-bottomed wells, 4x6 format, made of clear PET-plastic, printed lines between wells, chemically

resistant to all HiSafe-cocktails, max. volume 1 mL/well. Sealed with tape type 1450-461 or 1450-462. Counted in cassette type 1450-102.

1450-408 24-well Heat-sealable Sample Plate (25/box): 24 flat-bottomed wells, 4x6 format, made of clear PET-plastic, printed lines between wells, chemically resistant to all HiSafe-cocktails, max. volume 1 ml/well. Sealed with tape type 1450-461 or 1450-462. Counted in a cassette 1450-102.

6005040 96-well IsoplateTM (50/box): 96 flat-bottomed wells, 8x12 format, made of clear polystyrene wells with a rigid white frame, max. volume 350 μ L /well. For aqueous samples (e.g. SPA) or for samples with SuperMix cocktail 1200-439. Sealed with tape type 1450-461 or 1450-462. Counted in cassette type 1450-105.

6005050 96-well Tissue Culture Treated Black Isoplate (50/box): Black 96-well microplate with clear wells to allow reading from below (top and/or bottom reading). The plate is sterile and has a special surface treatment. It is ideal for cell attachment and proliferation, and suitable for use with a microscope. It comes with a lid and is individually wrapped. Sealed with tape type 1450-461 or 1450-462. Counted in cassette type 1450-105.

6007290 OptiPlateTM**-384 White Opaque Microplate** (50/box): White 384-well microplate with optimized well design and high quality plastics to enhance signal reflectivity at low backgrounds and crosstalk. Sealed with tape type 1450-461 or 1450-462. Counted in cassette type 1450-130.

6007279 OptiPlate-384 Black Opaque Microplate (200/box): Black 384well microplate providing reduced autofluorescence and low background signals. The high-polished, round-cornered wells are more conical in shape, which improves the reflectance signal but maintains a working volume of 10 - 75μ L per well. The plate is ideal for fluorescent assays like TRF and Fluorescence Polarization. Sealed with tape type 1450-461 or 1450-462. Counted in cassette type 1450-130.

6005290 OptiPlate-96, White Opaque Microplate (50/box): White opaque 96-well microplate providing enhanced light reflection properties for counting luminescence assays, while for liquid scintillation counting with MicroScint[™] cocktails, the plate combines high counting efficiency with reduced crosstalk. Sealed with tape type 1450-461 or 1450-462. Counted in cassette type 1450-105.

6005279 OptiPlate-96 Black Opaque Microplate (200/box): Black 96 well microplate providing reduced autofluorescence and low background signals. The plate is ideal for TR-FRET (LANCE) assays, and can also be used for high-signal luminescent assays to reduce unwanted crosstalk. Sealed with tape type 1450-461 or 1450-462. Counted in cassette type 1450-105.

6005070 96-well Sterile IsoplateTM (50/box): 96-well flat-bottomed, clear polystyrene wells with a rigid white frame, max. volume 350 μ L /well. For aqueous samples (e.g. SPA) or for samples with SuperMix cocktail 1200-

439. Sealed with tape type 1450-461 or 1450-462. Counted in cassette type 1450-105. Lid included.

6005590 96-well High Binding IsoplateTM for plate coating (50/box): 96well flat-bottomed, clear polystyrene wells with a rigid white frame, max. volume 350 μ L /well. For aqueous samples (e.g. SPA) or for samples with SuperMix cocktail 1200-439. Sealed with tape type 1450-461 or 1450-462. Counted in cassette type 1450-105.

6005340 ScintiPlateTM (50/box) 96-well plate format. Made of scintillation plastics, max. volume 300 μ L/well. For immobilized samples or aqueous samples, no scintillation cocktail needed. Sealed with tape type 1450-461 or 1450-462. Counted in cassette type 1450-105.

6005390 Sterile ScintiPlateTM (50/box) 96-well plate format. Made of scintillation plastics, max. volume 300 μ L/well. For immobilized samples or aqueous samples, no scintillation cocktail needed. Sealed with tape type 1450-461 or 1450-462. Counted in cassette type 1450-105. Lid included.

SMP200 FlashPlate (50/box) 96-well plate format. Coated with a scintillant, max. volume 300 μ L/well. For immobilized samples or aqueous samples, no scintillation cocktail needed. Sealed with tape type 1450-461 or 1450-462. Counted in cassette type 1450-105.

SMP400 FlashPlate (20/box) 384-well plate format. Coated with a scintillant, max. volume 60 μ L/well. For immobilized samples or aqueous samples, no scintillation cocktail needed. Sealed with tape type 1450-461 or 1450-462. Counted in cassette type 1450-130.

6006633 LumaPlate (100/box) 96-well plate format. Scintillant dried on the bottom of the plate, max. volume 100 μ L/well. For immobilized samples or aqueous samples, no scintillation cocktail needed. Sealed with tape type 1450-461 or 1450-462. Counted in cassette type 1450-105.

6005630 Deep Well LumaPlate (50/box) 96-well plate format. Scintillant dried on the bottom of the plate, max. volume 300 μ L/well. For immobilized samples or aqueous samples, no scintillation cocktail needed. Sealed with tape type 1450-461 or 1450-462. Counted in cassette type 1450-105.

6005290 OptiPlate (50/box) 96-well plate format. Opaque white plate, max volume 300 μ L. For non-sterile luminescence counting. Sealed with tape type 1450-461 or 1450-462. Counted in cassette type 1450-105.

6007290 OptiPlate (50/box) 384-well plate format. Opaque white plate, max volume 75 μ L. For non-sterile luminescence counting. Sealed with tape type 1450-461 or 1450-462. Counted in cassette type 1450-130.

6005500 High Binding OptiPlate (50/box) 96-well plate format. Opaque white plate, max volume 300 μ L. For ELISA luminescence counting. Sealed with tape type 1450-461 or 1450-462. Counted in cassette type 1450-105.

6005620 High Binding OptiPlate (50/box) 384-well plate format. Opaque white plate, max volume 75 μ L. For ELISA luminescence counting. Sealed with tape type 1450-461 or 1450-462. Counted in cassette type 1450-130.

6005680 Sterile CulturPlate (50/box) 96-well plate format. Opaque white plate, max volume 300 μ L. For sterile luminescence counting. Counted in cassette type 1450-105. Lid included.

6007680 Sterile CulturPlate (50/box) 384.well plate format. Opaque white plate, max volume 75 μ L. For sterile luminescence counting. Counted in cassette type 1450-130.

1450-603 VisiPlate-24 TC (14/box) White 24-well microplate with lid. Clear bottom, sterile and tissue culture treated,

Filtermats

1450-421 Printed Filtermat A (100/box): 96-position glassfibre filter mat with printed pattern. For harvested cell and receptor samples. Scintillant to be used: Betaplate Scint 1205-440 or MeltiLex B/HS 1450-442. Placed in a sample bag type 1450-432. Counted in cassette type 1450-104.

1450-422 Filtermat A (100/box): 24-position glass -fibre filter mat with printed pattern. For harvested cell and receptor samples. Scintillant to be used: Betaplate Scint 1205-440 or MeltiLex A 1450-441. Place in a sample bag 1450-432. Counted in cassette type 1450-116.

1450-423 Printed Nylon Membrane (50/box): 96-position nylon membrane with printed pattern; for DNA samples. Scintillant to be used: Betaplate Scint 1205-440 or MeltiLex A 1450-441. Placed in sample bag type 1450-432. Counted in cassette type 1450-104.

1450-424 Filtermat B (50/box): 24-position filtermat with printed pattern made of glassfibre. For harvested or pipetted samples. Scintillant to be used: Betaplate Scint 1205-440 or MeltiLex B/HS 1450-442. Place in a sample bag 1450-432. Counted in cassette type 1450-116.

1450-521 Printed Filtermat B (50/box): 96-position glassfibre filter mat with printed pattern. For harvested receptor or cell samples. Scintillant to be used: Betaplate Scint 1205-440 or MeltiLex A 1450-441. Placed in sample bag type 1450-432. Counted in cassette type 1450-104.

1450-522 DEAE Filtermat (100/box): 96-position filter mat with printed pattern made of glassfibre, containing positively charged groups. For harvested or pipetted (negatively charged) samples. Scintillant to be used: Betaplate Scint 1205-440 or MeltiLex A 1450-441. Placed in sample bag type 1450-432. Counted in cassette type 1450-104.

1450-523 P30 Filtermat (100/box): 96-position filter mat with printed pattern made of glassfibre, containing negatively charged groups. For harvested or pipetted (positively charged) samples. Scintillant to be used: Betaplate Scint 1205-440 or MeltiLex A 1450-441. Placed in a sample bag 1450-432. Counted in a cassette type 1450-104.

Plastic Bags

1450-431 Waste Bag (100/box): Waste bag for used sample plates. Chemically resistant to safe cocktails. Closed with a heatsealer (1295-012).

1450-432 Sample Bag (100/box): Sample bag for filters 1450-421 and 1450-423. Chemically resistant to safe cocktails. Closed with a heatsealer (1295-012).

Scintillation Products

1450-441 MeltiLex A (100/box): Meltable solid scintillator for filters 1450-421 and 1450-423 or other filters. Melting temperature +60..+120°C.

1450-442 MeltiLex B/HS (50/box): Meltable solid scintillator for filters 1450-521, 1450-424 and other thick filters. Melting temperature +60..+120°C.

1200-439 OptiPhase SuperMix (5L/bottle): Scintillation cocktail to be used in microtiterplates. Mixes well in small volumes.

1200-436 OptiPhase Hisafe 2 (5L/bottle): Scintillation cocktail to be used with non-aqueous samples.

1205-440 Betaplate Scint (5L/bottle): scintillation cockatail to be used with samples harvested onto dry filter membranes. Ideal for use with samples in organiz solutions.

Cassettes

Cassettes are made of special-grade polycarbonate plastic containing a high amount of white pigment to produce a very high degree of reflectivity (about 90 %) while eliminating optical crosstalk between wells. Measurement is made through the cassettes (pat. pend.).

Cassette 1450-101. This has 96 sample holes with a diameter of 7.8mm. The cassette is used when counting 1450-401 (or equivalent) 96 well sample plates.

Cassette 1450-102. This has 24 sample holes with a diameter of 13.2 mm (see the figure). This cassette is used when counting 1450-402 (or equivalent) 24-well sample plates.

Cassette 1450-104. This is a two piece cassette with 96 sample holes. This cassette is used when counting filtermats. The filtermat in a sample bag is placed between the base plate and the cover plate.

Cassette 1450-105. This has 96 sample holes. This cassette is used when counting 6005040 or equivalent sample plates.

Cassette 1450-106. This has 96 sample holes. This cassette is used when counting e.g. Millipore MultiScreen[™] Filtration plates or equivalent sample plates.

Cassette 1450-107. This is a two piece cassette with 24 sample holes. The cassette is used when counting Skatron filtermats cut into four pieces. The filtermat in a sample bag is placed between the two plates of the cassette.

Cassette 1450-110. This is an open-based cassette. The cassette is used when counting 24-well culture plates, e.g. Costar plates (or equivalent).

Cassette 1450-111. This is an 8 x 12 luminescence filter cassette.

Cassette 1450-116. This is a two piece cassette with 24 sample holes. This cassette is used when counting filtermats. The filtermat in a sample bag is placed between the base plate and the cover plate.

Cassette 1450-117. This is a two piece cassette with 24 sample holes. This cassette is used when counting 4 mL sample vials. The vials are placed between the base plate and the cover plate.

Cassette 1450-118. This is a two piece cassette with 96 sample holes. This cassette is used when counting e.g. ³²P-labeled dot blot samples from filtermats such as 1450-423 Nylon membrane. No scintillator needs to be added to the filtermat allowing reprobing assays. The filtermat in a sample bag is placed between the base plate and the cover plate having solid scintillator in sample positions.

Cassette 1450-119. This cassette is used with 24-well plate VisiPlates. VisiPlates have clear bottom and white walls on the wells. If fully clear 24-well plates are used, one should remember to use also 1450-109 inserts to prevent the crosstalk. Therefore the usage of crosstalk-free VisiPlates is highly recommended.

Cassette 1450-130. This has 384 sample holes. This is used when counting 384-well sample plates.

Cassette 2450-2050. This is a cassette for a 384-well Millipore Multiscreen plate

Cassette 2450-2060. This is a 384-well plate cassette for robotic loading

Cassette 2450-2070. This is a 96-well plate cassette for robotic loading

Adapters and inserts

Adapter 1450-108. These adapters are used when counting Eppendorf tubes (or equivalent). The adapters are placed on a 1450-102 cassette; a bottom tape is used if needed.

Inserts 1450-109. These inserts are used when counting 24 well culture plates. The inserts are placed in the sample wells in order to reduce crosstalk between the wells.

ID Products

1450-451 ID Support Plate: Support plate for ID labels. Placed onto a counting cassette.

2451-4010 ID Labels (10 sheets/box): Labels for identifying Protocol and Cassette number and Function code. Fixed on a Support Plate 1450-451.

Tapes

1450-461 Sealing Tape (100/box): Sealing tape for sample plates, permanent.

1450-462 Removable Sealing Tape (100/box): Sealing tape for sample plates; removable.

1450-465 Printed Sealing Tape (100/box): Sealing tape for sample plates, printed.

Other Consumables

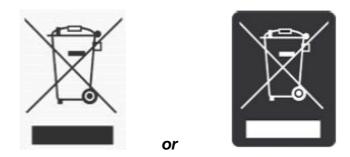
1450-481 Support Frame (25/box): Support frame for sample plates type 1450-401, 1450-402 and 1450-408. Removed before counting. This support frame can be used to keep the flexible plate in a strict format eg. during the shaking in a plate shaker.

1450-486 MicroBeta² **Starter kit, MicroPlates**: Contains the MicroBeta² sample plates (5 of each), and tapes.

1450-487 MicroBeta² **Starter kit, Filters, Membranes and Solid Scintillators**: Contains the MicroBeta² filters, membranes, MeltiLex-sheets, Sample bags and Waste bags.

Chapter 7 Waste disposal

WEEE Instructions for PerkinElmer Products



A label with a crossed-out wheeled bin symbol and a rectangular bar indicates that the product is covered by the Waste Electrical and Electronic Equipment (WEEE) Directive and is not to be disposed of as unsorted municipal waste. Any products marked with this symbol must be collected separately, according to the regulatory guidelines in your area.

The objectives of this program are to preserve, protect and improve the quality of the environment, protect human health, and utilize natural resources prudently and rationally. Specific treatment of WEEE is indispensable in order to avoid the dispersion of pollutants into the recycled material or waste stream. Such treatment is the most effective means of protecting the customer's environment.

Requirements for waste collection, reuse, recycling, and recovery programs vary by regulatory authority at your location. Contact your local responsible body (e.g., your laboratory manager) or authorized representative for information regarding applicable disposal regulations. Contact PerkinElmer at the web site listed below for information specific to PerkinElmer products.

Web address: http://las.perkinelmer.com/OneSource/Environmental-directives.htm

Customer Care:	1-800-762-4000 (+1) 203-925-4602	(inside the USA) (outside the USA)
	0800 40 858 0800 90 66 42	(Brussels) (Monza)

Products from other manufacturers may also form a part of your PerkinElmer system. These other producers are directly responsible for the collection and processing of their own waste products under the terms of the WEEE Directive. Please contact these producers directly before discarding any of their products.

Consult the PerkinElmer web site (above) for producer names and web addresses.



Chapter 8 Abbreviations and acronyms

Abbreviations and acronyms used

	•
AQP(I)	Asymmetric quench parameter of the isotope spectrum
AQP(I)%	Theoretical percentage error of <u>AQP(I)</u>
AS	Value of <u>as</u> say ID for the current plate
CA	Value of <u>ca</u> ssette ID for the current plate
CCPM1	<u>C</u> orrected count rate (<u>c</u> ounts <u>per minute</u>) for isotope <u>1</u>
CCPM1%	Theoretical percentage error for CCPM1
CCPM2	<u>C</u> orrected count rate (<u>c</u> ounts <u>per minute</u>) for isotope <u>2</u>
CCPM2%	Theoretical percentage error for CCPM2
CCPM3	<u>C</u> orrected count rate (<u>c</u> ounts <u>per minute</u>) for window <u>3</u>
CCPM3%	Theoretical percentage error for CCPM3
CCPS1	<u>C</u> orrected count rate (<u>c</u> ounts <u>per second</u>) for isotope <u>1</u>
CCPS2	<u>C</u> orrected count rate (<u>c</u> ounts <u>per second</u>) for isotope $\underline{2}$
CCPS3	<u>C</u> orrected count rate (<u>c</u> ounts <u>per second</u>) for window <u>3</u>
CNO	<u>C</u> assette order <u>n</u> umber
COUNTS1	Counts for isotope <u>1</u>
COUNTS2	Counts for isotope 2
COUNTS3	Counts for window 3
CPM_AQP	<u>AQP(I)</u> count rate (<u>c</u> ounts <u>per minute</u>)
CPM_C	Coincidence count rate (counts per minute)
CPM_NC	<u>Non-coincidence count rate (counts per minute)</u>
CPM1	Count rate (counts per minute) for isotope 1
CPM1%	Theoretical percentage error for CPM1
CPM2	Count rate (counts per minute) for isotope 2
CPM2%	Theoretical percentage error for CPM2
CPM3	Count rate (counts per minute) for window 3
CPM3%	Theoretical percentage error for CPM3
CPS1	Count rate (counts per second) for isotope 1
CPS2	Count rate (counts per second) for isotope 2
CPS3	Count rate (counts per second) for window 3
CT	<u>C</u> assette <u>type</u>
CTIME	Dead time corrected <u>counting time</u> in seconds
D	Detector number
DATE	Current date
DCCPM1	Absolute theoretical error for <u>CCPM1</u>
DCCPM2	Absolute theoretical error for <u>CCPM2</u>
DCCPM3	Absolute theoretical error for <u>CCPM3</u>
DDPM1	Absolute theoretical error for <u>DPM1</u>
DDPM2	Absolute theoretical error for <u>DPM2</u>
DLCPS	Absolute theoretical error for <u>LCPS</u>
DPM1	Activity (disintegration per minute) for isotope 1
DPM1%	Theoretical percentage error for DPM1
DPM2	Activity (disintegration per minute) for isotope 2

DPM2%	Theoretical percentage error for DPM2
DPMM	<u>DPM</u> out of range <u>m</u> onitor
EFF1	Counting <u>efficiency</u> for isotope <u>1</u>
EFF2	Counting <u>eff</u> iciency for isotope <u>2</u>
EFF3	Extra window 3 counting efficiency
ETIME1	Elapsed time in hours for isotope 1 from zero time 1
ETIME2	Elapsed time in hours for isotope 2 from zero time 2
FLAG	Termination <u>flag</u>
FU	Value of <u>function</u> ID code for the current plate
ISEP	Isotope spectrum end point
ISEP%	Theoretical percentage error for ISEP
LCPS	Luminescence corrected count rate (counts per second) divided by 100
LCPS%	Theoretical <u>percentage</u> error for <u>LCPS</u>
	LCPS out of range monitor
IDSHORT	Plate <u>id</u> entifier (<u>short</u> right hand side of the plate)
IDLONG	Plate <u>id</u> entifier (<u>long</u> bottom side of the plate)
POS	Sample position on microtitration plate
PR	Protocol number used
RESP1	Response (CCPM1 or LCPS)
RESP1%	Theoretical percentage error for <u>RESP1</u>
RESP2	Response (CCPM2)
RESP2%	Theoretical percentage error for RESP2
RP	Repeat number of the current measurement
S	Spectrum half
SEQ	Running sequential sample number
SH	Shelf number
SPECTRA	Plot <u>spectra</u>
SQP(I)	Spectrum quench parameter of isotope spectrum
SQP(I)%	Theoretical percentage error for <u>SQP(I)</u>
STIME	Counting start time
TD	Temperature of upper detector block
TIME	Current time

Chapter 9 Installation

Installation instructions

You are advised to read through the whole of this section before starting to unpack the instrument.

Note! Except for where noted these instructions are valid for both 2450 MicroBeta² and 2460 MicroBeta² LumiJET.

Environment

Although normal clean laboratory conditions are usually quite satisfactory as an operational environment it is useful to take the following points into consideration.

If possible a separate room should be provided for the MicroBeta² unit as this allows the best control over the immediate environment. Ventilation in the room should be adequate for all conditions of use, the temperature should be reasonably constant at about 22°C, relative humidity should not be excessive, and direct sunlight should not be able to reach the instrument. It is also important that the various nuclides are stored well away from the instrument in another room. Only those radioactive samples that are actually measured should be in the laboratory at any time in order to keep the background at a low level.

Electric power

Three electrical outlets each having a protective earth should be available, with, if possible, a separate power line for the instrument itself having an isolation switch and a fuse box. If excessive fluctuations in the mains voltage are anticipated, a mains stabilizer may be necessary.

Lifting the instrument

The weight of the instrument is over 80 kg, thus three or four persons are needed for its lifting and transportation. The instrument must be lifted by supporting it under its bottom plate. 32-shelf models can also be lifted using the back panel when removing the instrument from its pack. The 32-shelf model should be installed immediately onto its table to secure safe moving of the instrument. There is adequate finger room between instrument's bottom plate and the table.

Warning! Do not attempt to lift the instrument by placing your fingers under its feet.

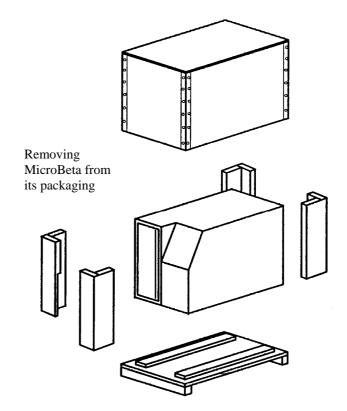
Unpacking

Cut off and remove the binding bands.

Open the clinching nails and lift the cover off from the base of the package.

Unpack all units and accessories and check them according to the packing list also noting any possible transport damage.

Open the lower clinching nails and remove the packing around the main instrument.



Lift the instrument up from the bottom of the package.

Move the instrument to its place of operation.

Checking the mains voltage setting

Measure and note the mains voltage at the outlets to be used. The voltage should be within the range specified in Chapter 6, Specifications, Power requirements (see page 52).

Insert the 4A battery fuse in its holder into the socket at the rear of the instrument.

Connecting up the counter and peripherals

Connect the mouse, keyboard and display to the instrument. Connect the optional printer to a USB port of the instrument.

See the additional section at the end of this chapter for information about installation of the injector system to MicroBeta² LumiJET.

Plug in the power cables for instrument and the optional printer.

Start-up

1. Switch on the printer.

2. Switch on the instrument.

3. After about 1 minute a normal Windows XP view will appear on the screen.

4. Check the date and time from the Windows system parameters and set them to the current values if needed.

5. Double click the MicroBeta² Workstation icon.

6. If Enhanced Security option is installed, the software will ask for the Administrator user name and password. For this first time the Administrator user name is MBWIWAdmin and the password is MBWIWAdmin. To learn how to set up additional users, refer to the relevant section under Installation in the User Manual.

7. **Ready** is displayed at the bottom of the Workstation Window and the counter is ready for operation.

Test settings

For checking the performance of the instrument, run the detector efficiency normalization protocol 89 with the 1450-471 detector normalization standard plate and 1450-105 cassette supplied with the instrument. Use an appropriate ID label to verify proper operation of the ID reader. As a background plate, use one Isoplate 1450-414 delivered with the instrument. Place the background plate in shelf number 1 and the normalization sample in shelf number 16. Check that the results are as good as those delivered with the instrument.

Setting the printer

Printer connection

A printer can be either connected to a USB port of instrument or a network printer can be used. The definition of the printer is selected under File PrintSetUp. If the printer is connected to the instrument, the printer driver needs to be installed on the Workstation's Windows.

Installation of the Injector System to MicroBeta² LumiJET

Perform a normal MicroBeta² installation (mechanical check and check of efficiencies and background). Switch the instrument off and attach the injector system to the instrument:

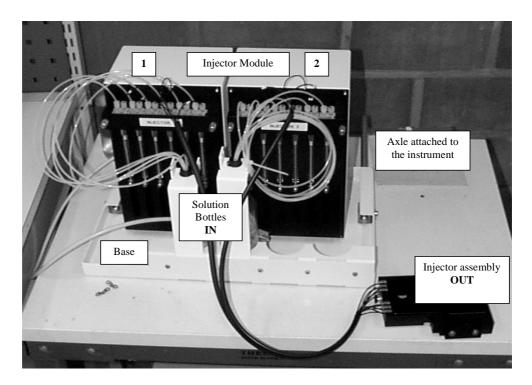
Caution! Always power down the instrument when connecting or disconnecting pumps.

Attach the injector modules to the base (two screws at the bottom)

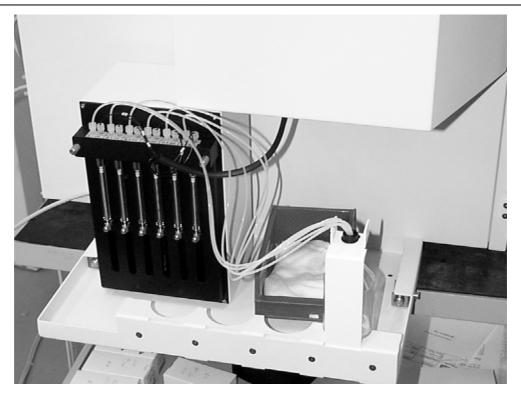
Attach the support pieces for the solution bottles

Attach the transparent tubing of the solution bottles to the IN connectors of the pumps

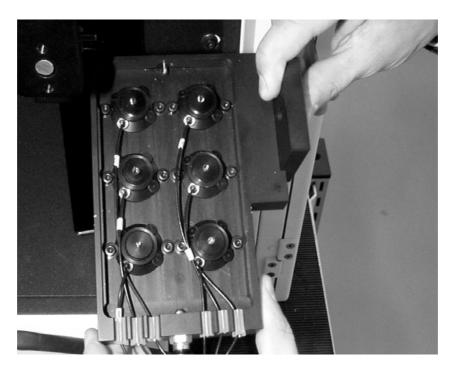
Attach the black injector adapter tube No. 1 to the out connector of pump No. 1 and tube No. 2 to the out connector of pump No. 2 etc. If there is more than one injector module the units are numbered.



Attach the whole injector system to the left side of the MicroBeta² JET by sliding the axles into holes in the support pieces attached to the instrument.



Open the lid of the injector adapter module and check the tubing (see picture below). Systems with two injector modules have a second tube on the left side connector



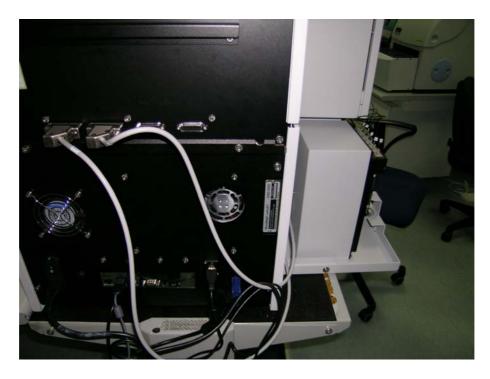
Cut the tie which is attached to the arm for the injector assembly Attach the injector assembly to the arm (see picture below).

Installation instructions



The picture above shows the view from inside when the tubing is fitted.

Connect the cable from each injector to the appropriate connector at the rear of MicroBeta² LumiJET (see picture below).



Boot the system up.

After initializing and priming you should dispense at least once the volume to be used in the assay. The dispensing volumes and alignment to the sample plate can be tested by running a counting protocol. Counting protocol parameters are set for the dispensing speed and volumes for the selected modules.

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