

Filter counting applications with the MicroBeta² microplate counter.

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Introduction

The MicroBeta²® microplate counter is a multi-detector instrument for liquid scintillation and luminescence counting. It is available in 1-, 2-, 6- and 12-detector versions. The 1- and 2-detector models are equipped with 24- and 96-well counting capabilities and by default the 6- and 12-detector units with 96- and 384-well support. The LumiJET version of MicroBeta² provides dispensing capabilities for kinetic luminescence applications, such as Aequorin-based GPCR assays, with two dispensers per detector for up to 384-well plates and twelve detectors.

This application note presents a cell proliferation (thymidine incorporation) assay using a cell harvesting method and filter counting on the MicroBeta².

Background

The cell proliferation assay with ³H-Thymidine incorporation is one of the most reliable and popular filter counting applications. There are several alternative non-radioactive methods for thymidine incorporation; however, they do not correlate directly with DNA synthesis. Additionally, they contain many additional assay steps compared to the ³H-Thymidine filtration assay. These alternative assays, such as cell viability assays, only measure the increase or decrease of viable cell number, not the cell division rate itself. Thus, in a viability assay, the response does not change when the cell death rate is the same as the division rate.



A thymidine incorporation assay can be performed in several ways using the MicroBeta² microplate counter. In this application note, a cell harvesting method using a GF/C type glass fiber Filtermat A or a GF/C Unifilter[®] plate is described. Optionally, the assay can be performed directly in a tissue culture grade Isoplate[™] with adherent cells.

Receptor-ligand binding assay with radioligands is another established reference method. The compound behavior of a tritiated ligand is the same as the unlabeled ligand; thus the method provides accurate and natural binding affinities. The GF/C type filter material can be used in receptor-ligand binding studies.

Both Unifilter[®] plates and Filtermats are also available as GF/B versions. By using a GF/B type glass fiber filter, which is twice as thick as a GF/C filter, the capturing efficiency of the receptor prepartate is increased. Receptor-ligand studies often involve an organic soluble radioligand. In these cases, MeltiLex[®] solid scintillator is used with the Filtermats to prevent activity from spreading across the Filtermat. The MeltiLex can be applied to a dried filter by melting it on a 90°C hot plate. The Unifilter[®] plate contains discrete filter discs, which are separated from each other in the manufacturing process of the plate and does not require any special scintillator approach.

Counting Unifilter[®] plates on MicroBeta²

Each MicroBeta² detector is comprised of two photo-multiplier tubes (PMTs) in conventional coincidence circuitry. The coincidence mode is very efficient and has an excellent signal-to-noise ratio for normal scintillation counting. The normal coincidence mode can be used with clear bottom plates or mini-vials. It can also be used with the filtermats, which become translucent after scintillator addition. Alternatively, just the upper PMT can be used for luminescence detection or liquid scintillation counting of opaque white microplates.

To optimize counting, the Unifilter[®] plate is measured only with the upper PMT using the "Top read" assay type, instead of in coincidence mode. A white seal can be added onto the bottom of the plate to reflect more light and increase the counting efficiency.

Generally, using a single photo-multiplier tube instead of a coincidence pair is more challenging because of the high background level of a single PMT. With MicroBeta², the "Top read" upper tube detection performance is improved by using Time-Resolved LSC counting mode (TR-LSC).

This method benefits a "slow scintillator" such as MicroScint[™] cocktail or yttrium silicate in Lumaplate[™] microplates. When the decay time of the scintillation event is extended by the slow scintillator, individual photons in a scintillation event become detectable. If a counting event contains only a single pulse, it is most probably produced by background noise of the PMT. To register a count in High sensitivity TR-LSC mode, at least three pulses are required within the 200 ns time window, resulting in low background and good counting efficiency. The principle of TR-LSC is shown in Figure 1.

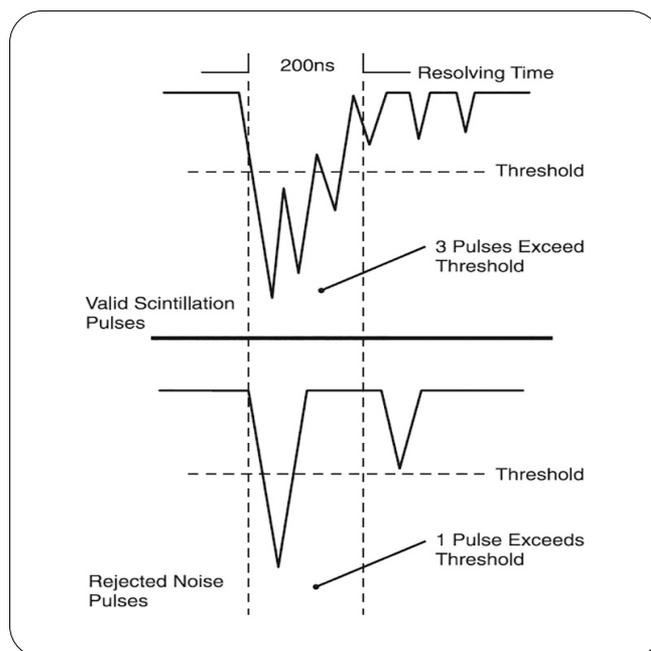


Figure 1. In High sensitivity TR-LSC mode, at least three individual pulses must cross the threshold to register a count. This prevents any randomly occurring single background pulses produced by thermal noise to trigger a count.

FilterMate harvester

The FilterMate harvester is very robust and simple to use. It supports all MicroBeta² sample formats. With an upgrade kit, it is possible to later switch between formats by replacing the interchangeable head. (see table 1.)

Table 1.

Product	Part number	Description
FilterMate Harvester	D961962	Harvester-96, Filtermat
FilterMate Harvester	D961241	Harvester-24, Filtermat
FilterMate Harvester	C961961	Harvester-96, Unifilter
Adapter Kit	7601415	Kit, Filtermat 96
Adapter Kit	7601414	Kit, Filtermat 24

Filter format

In receptor-ligand applications, the filter spot size plays an important role. It is possible to estimate the achieved assay count level based on the receptor concentration and specific activity of the radioligand. For example, if the receptor concentration is 1 pmol per mg and the specific activity of the radioligand is 48 Ci/mmol, a spot with 100 µg collected protein at a saturated binding level would contain 10560 DPM. This would result in 2323 CPM using a FiltermatA and MeltiLex at a 22% counting efficiency.

The spot size of the filter defines how much membrane prepartate can be captured per spot. An excess amount of protein per filter will block the membrane and prevent the wash steps. A 96-well filter has a spot diameter of 6.5 mm, which equals to 33 mm². A maximum load on this is between 100 µg and 200 µg of protein. The 24-well has a spot size of 95 mm² and captures a maximum load of 600 µg, resulting in around three times higher signal levels for low concentration receptors

Drying times

Drying is a very important step when the highest counting efficiency is required. (see table 2.) The signal is also instant and stable after scintillator addition. In an automated system, the Unifilter® plates can be measured moist, but then a larger volume of water mixing cocktail such as MicroScint 40 must be used. The double thickness filters require extended drying times.

Table 2.

Product	Drying method	Drying time
Filtermat A	Microwave	2 minutes
Filtermat A	Oven, 90°C	10 minutes
Filtermat A	Hot plate, 90°C	10 minutes
Unifilter GF/C	Hot plate, 50°C	100 minutes
Unifilter GF/C	Oven, 50°C	70 minutes

Methods

A traditional cell proliferation study was performed. The cells were washed and precipitated with a TCA treatment after the ³H-Thymidine labeling. The precipitate was dissolved in NaOH, then neutralized with HCl. A previous study showed that the TCA treatment can be omitted all together, and it is enough to wash the cells and loosen adherent cells with trypsin prior to harvesting. This makes the harvesting experiment simple and quick to perform.

For the harvesting experiment, 10 mL of Jurkat suspension cells (human white blood cells) in a concentration of 1 million cells per mL were treated for eight hours with 2 µCi/mL ³H-Thymidine (Revvity, NET355250UC). In every cell division, DNA synthesis incorporates tritiated thymidine into the cell DNA. A 1:1 dilution series of the cells was prepared starting from 100,000 cells per well. On a 96-well plate, 100 µL of each dilution was pipetted in four replicates. One plate was harvested on a Filtermat A (1450-421) using a Filtermatcapable FilterMate harvester and the other plate on a Unifilter® 96 GF/C (6005174) with a Unifilter®-capable FilterMate harvester. Harvesting consisted of a pre-wet step, aspiration of the cells through the filter and six wash steps (aspiration of filled buffer wells). In thymidine incorporation harvesting, pure water was used as wash buffer.

The Filtermat was dried for 10 minutes on a 90°C hotplate and the Unifilter® plate in a 50°C oven for 2 hours. The Filtermat was placed in a sample bag (1450-432) and 5 mL of Betaplate Scint (1205-440) was added and spread across the filter. The bag was sealed with the Bag Heat Sealer (1295-012) and the filter was placed in a filter cassette (1450-514) for counting.

A white seal was attached into the bottom of the Unifilter® plate and 25 µL per well MicroScint O cocktail (6013611) was added. The plate was sealed with Top Seal-A™(6005185) and placed in a 96- plate cassette (1450-105) for counting.

The Filtermat A was counted with the preset filter counting protocol one minute per sample. The Unifilter® was counted with a preset Top read protocol for 1 minute per sample with the "High sensitivity" TR-LSC setting. The settings are shown in Figure 2.

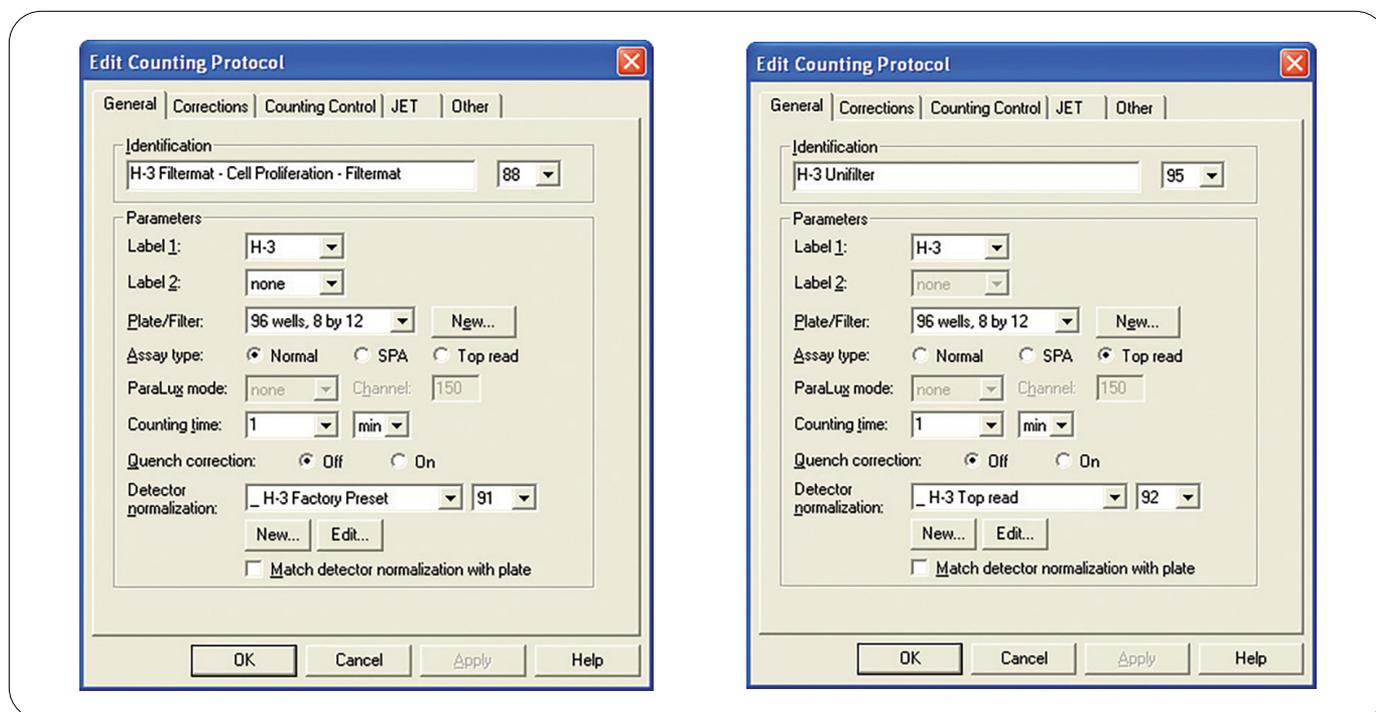


Figure 2. **Preset H-3 Filtermat and Unifilter® counting protocols.** For the Filtermat, a normal coincidence assay mode is used with a one minute counting time. The Unifilter requires a Top read assay mode with High sensitivity TR-LSC setting. In this mode, a background subtraction is performed based on the detector normalization data.

Results

The results show that both filtering systems have a linear response across a wide range of cell number. Filtermat A was measured with a normal coincidence counting method using Betaplate Scint scintillation fluid, which has a 45% counting efficiency for H-3. Based on this information, the Unifilter® counting efficiency using MicroScint O and a white adhesive tape in the bottom of the plate can be estimated to be 34% for H-3. For both filter types, the average coefficient of variation (CV%) was less than 3% for samples producing over 4000 counts per minute (CPM) and less than 10% for the samples above 1000 CPM. The results for the dilution series are shown in Figure 3.

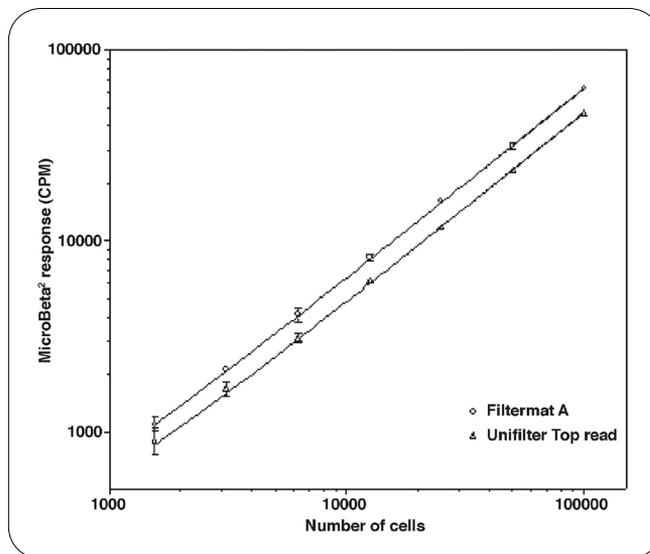


Figure 3. **³H-Thymidine labeled Jurkat cells harvested on a glass fiber filter** and measured with the MicroBeta² Microplate Counter. Both Filtermat A and Unifilter plate had a very good linear correlation (R=0.9999) over a wide range of cell numbers.

Summary

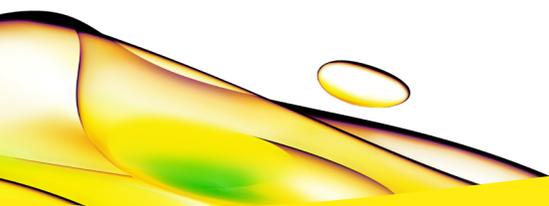
The MicroBeta² Microplate Counter is a very versatile and high performance reader for applications requiring a filtering step. Both the Filtermat and a filter-plate based approach provide equal assay performance for cell proliferation assays. Differences can be found in other usage and handling preferences, listed below.

Filtermat:

- Available in 96- and 24-well formats
- Excellent counting efficiency and low background
- Low cost structure
- Fast drying options: 2 minutes in a microwave
- Solid waste with the MeltiLex scintillator
- MeltiLex solid scintillator for organic soluble radioligands
- Only one scintillator addition step per 96 samples
- Small scintillator consumption: 5 mL per one Filtermat A
- Possibility to cut the spots into vials
- Requires a sample bag with liquid scintillator

Unifilter plate:

- Available in 96- and 384-well formats
- Easy to handle: it is a plate
- Automation and stacker compatible
- Low risk of cross contamination due to separating well structure
- MicroScint scintillation cocktail enables TR-LSC counting method
- Low scintillator consumption per sample: 25 μ L per 96-well



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