Application Notes

Counting FlashPlates on the Wallac MicroBeta®

FlashPlates and ScintiPlates are popular platforms for running a variety of radiometric screening assays. The Wallac MicroBeta effectively measures both plate types.

MicroBeta detectors comprise two photomultiplier tubes, one above the sample and one below.

FlashPlate is a solid white plate and so MicroBeta measurements are conducted with the upper PM tube only. A comparison is made of MicroBeta with a Packard TopCount®, an instrument that employs single PM tube detectors.

ScintiPlate has clear wells so when measured on MicroBeta both upper and lower PM tubes are used. A comparison of MicroBeta performance is made when measuring the same assay with both FlashPlate and ScintiPlate.

APPLICATION I

A Reverse Transcriptase Assay was performed using a Streptavidin coated FlashPlate (SMP103). In this assay, a biotinylated DNA oligonucleotide acts as a primer for reverse transcriptase (RT) on an RNA template. The signal is generated by incorporation of labeled dTTP into the newly synthesized strand of DNA. Plates were read on both a TopCount and a MicroBeta, and as shown, the MicroBeta effectively reads with only the top PM tubes in use.

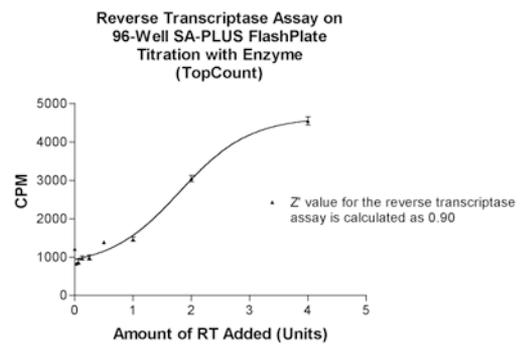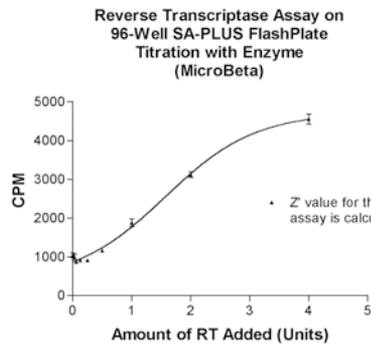## PerkinElmer™
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Figure 1 & 2: Reverse Transcriptase (NEI490, 10,000 units/mL) assay performance over a range of enzyme concentrations from 0.3 units to 4.0 units per reaction. The assay was conducted with 50 mM Tris-HCl (pH 8.0), 10 mM MgCl$_2$, 1 mM DTT, 500 units/mL Recombinant Rnasin™ ribonuclease inhibitor (Promega Corp.), 87.5 nM biotinylated 20-base DNA oligonucleotide primer (Keystone Lab), 67.03 nM 89-base RNA template, 43.75 µM of dATP, dCTP, and dGTP, 105 nM of [Methyl-1',2',-³H]dTTP (NET520A, 115 Ci/mmol). The reaction was initiated by the addition of the Reverse Transcriptase, and incubated at 37ºC for 60 minutes.
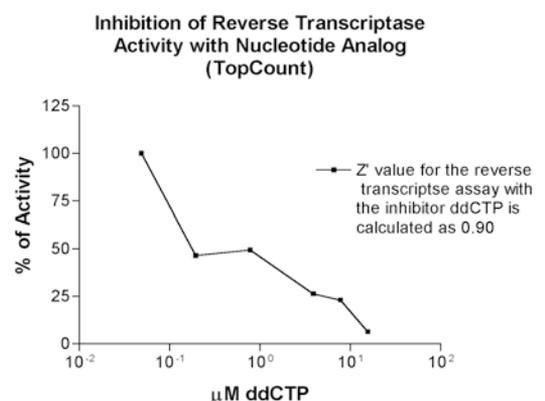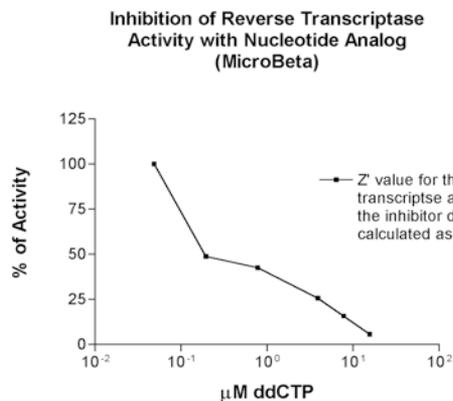

Figure 3 & 4: Reverse Transcriptase activity was inhibited by the addition of the nucleotide analog dideoxynucleotide (ddCTP). Reverse Transcriptase was added to each well followed by the addition of the inhibitor. The reaction was initiated by the addition of reaction mixture (primer / template complex, nucleotide, ribonuclease inhibitor, [³H] dTTP, DTT, MgCl$_2$, Tris-HCl pH 8.0). The assay was incubated at 37ºC for over night, and counted on both instruments.

APPLICATION II

To offer as diverse a range of applications as possible, there are a variety of FlashPlate and ScintiPlate coatings and surface treatments available.

The following experiment was devised to show that although using both PM tubes gives ultimate sensitivity, for example, when measuring adherent cells in ScintiPlates, MicroBeta still provides excellent performance with upper tube only counting.

[^{3}H]Biotin was titrated on both Streptavidin coated FlashPlates and ScintiPlates, then bound activity was read on the MicroBeta. The mass bound was determined by the differential between the known mass/DPM added and the unbound mass/DPM, using the known specific activity for the compound and the known efficiency of a LSC counter. The MicroBeta proved to read the opaque plate from the top as effectively as the clear well plate read from the top and bottom.

Streptavidin FlashPlate and ScintPlate Read on MicroBeta
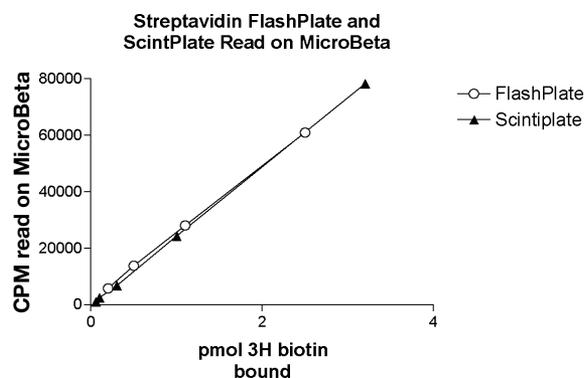
Instructions for counting FlashPlates on the MicroBeta. Software version 2.7

Create a new normalization

Select "Other" for the label

Select "Background Sample"

Click on "Extended"

Click on "PMT Usage"

Select "Upper" and click on "OK"

Change the window setting to:
H-3, 175 − 360
I-125, 175 − 530
C-14, 175 − 650
S-35, 175 − 650
P-33, 175 - 960

Click on OK

Click on "New" in the Plate/Filter section

Enter the name of the plate (i.e. FlashPlate). No other changes from the default protocol are necessary.

Important! Change the window setting after selecting the PMT usage. Otherwise the program will reset the window to 150 - 1024

Instructions for counting FlashPlates on the MicroBeta. Software version 3.0

Create a new normalization

Select "Other" for the label

Corrections Tab
Select "Background sample"

Other Tab
Select "Upper tube only"

Change the window setting to:
H-3, 175 – 360
I-125, 175 – 530
C-14, 175 – 650
S-35, 175 – 650
P-33, 175 - 960

Save the normalization protocol and then check the plate map. Two plates are required for FlashPlate normalization. The first one is an empty plate that is used for determining the background of each of the detectors. For example, a six-detector instrument will store six background values and subtract the appropriate value from each unknown - "detector specific background subtraction". The second plate has the active sample in G11 (96-well plate) or M22 (384-well plate). It is advisable to acquire at least 10,000 counts for each reading.

Next, create a counting protocol. The key selections, "Other", upper PM, and window setting must be identical to the normalization or the normalization will not be visible from within the counting protocol.

Now, when unknowns are selected, the CPM1 is the raw value and the CCPM1 is corrected for background and efficiency.

To avoid phosphorescence, both normalization plates and assay plates should be dark-adapted for a couple of minutes before counting.

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