TopCount Topics

TCA-001

Counting Non-Aqueous Samples in the TopCount® Microplate Scintillation and Luminescence Counter

Abstract

The microplate format has become a standard for many biological assays such as high throughput screening for drug discovery and radioimmunoassays. Productivity with these high volume procedures has been limited by the amount of sample handling required and low sample counting throughput. Now, however, microplate assays can be efficiently counted in microplates with the Packard TopCount Microplate Scintillation and Luminescence Counter. This paper summarizes TopCount's performance for counting samples in organic solvents or bound to the wells of microplates (in-plate assays) using MicroScint™-O scintillation cocktail, a special formulation for samples in organic solvents. Results from an enzyme inhibition assay and a microplate immunoassay obtained with TopCount are comparable to those obtained by conventional liquid scintillation and gamma counting.

Introduction

Enzyme inhibition assays are among the many tests used by pharmaceutical companies to screen new compounds for biological activity. These assays measure the inhibitory effect of test compounds on target enzymes such as HIV proteases, various kinases, RNA and DNA transcriptases and polymerases. Enzyme activity is often determined by measuring the conversion of a radiolabeled substrate to a product that can be separated from the substrate by chromatography or organic extraction. After the separation step, the ^{3}H- or ^{14}C-labeled products, often in organic solvents, are counted by liquid scintillation counting (LSC). The assays are often done in microplates, and it is highly desirable to quantitate the radiolabeled product in this format, especially when the product can be eluted directly into microplates. By quantitating in microplates, additional sample handling and liquid scintillation vials can be eliminated. In addition, sample and cocktail volumes, and the resulting waste disposal costs, can be reduced significantly. Keeping samples in microplates increases throughput and decreases processing time.

Immunoradiometric assays (IRMA) are often processed in microplates. Typically, this involves a sandwich assay in which the analyte binds to a first antibody which is immobilized in the wells of a microplate or a microplate strip. After washing, the analyte captured in the wells is detected by incubation with a second ^{125}I-labeled antibody. The ^{125}I label in the wells after further washing may be quantitated by transferring individual microplate wells to tubes in a gamma counter. Counting immunoassays directly in the microplates substantially decreases the sample handling.

To facilitate high throughput analysis Packard Instrument Company has introduced the TopCount Microplate Scintillation and Luminescence Counter for counting organic or aqueous samples in either 96-well or 24-well microplates. Specially designed, solvent resistant microplates, PicoPlates™, are provided for efficient scintillation counting. Polystyrene microplates are dissolved by many common opaque solvents. Up to 12 wells of a 96-well microplate can be counted simultaneously. TopCount can count an entire microplate in a fraction of the time it takes for conventional liquid scintillation or gamma counting. The available microplate stackers hold 20 or 40 plates for unattended, high throughput processing. The on-line data reduction capability of TopCount can further speed the process of turning raw data into final answers.

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In the experiments reported here, TopCount was used to count samples in organic solvents or on a solid support in the microplate format. The scintillation cocktail used was MicroScint-O, an environmentally safe formulation for counting samples in TopCount with most commonly used organic solvents such as heptane, ethyl acetate, methanol, and ethanol. Counting efficiencies were determined using ^{3}H and ^{14}C hexadecane for both the 96- and 24-well formats. To test the effects of sample load, varying volumes of samples in an organic solvent from an enzyme inhibition assay were counted in 96-well solvent resistant PicoPlates, and the results were compared to those obtained by conventional LSC. A TSH immunoradiometric assay was counted, and the results were compared to those obtained by gamma counting.

Experimental Methods

Counting efficiency was tested by adding ^{3}H hexadecane to three wells of a 96-well PicoPlate containing 250 µL of MicroScint-O. For the 24-well PicoPlate, ^{3}H was added to three wells containing 1.0 mL of cocktail. The same number of wells containing only cocktail were used for background determination. Similar experiments were carried out for ^{14}C. The plates were sealed with Packard's TopSeal™-P, a solvent resistant cover film, using the Packard MicroMate 496 Microplate Heat Sealer, and then mixed on a microplate shaker for 15 minutes. The samples were counted for five minutes each, under conditions optimized for liquid counting, on a TopCount with the VariPlate™ option which allows counting of either 96- or 24-well plates.

To test the effects of sample load, ^{3}H labeled samples in heptane solvent from an enzyme inhibition assay were counted using four different sample volumes. Aliquots of 25, 50, 100, and 150 µL in quadruplicate were pipetted into a solvent resistant 96-well PicoPlate with a multiple-tip pipetter. Total volumes were brought to 300 µL with MicroScint-O. The plates were sealed and counted as described above. An additional 50 µL aliquot was counted for five minutes in a Packard Tri-Carb® 2250CA liquid scintillation analyzer, using 5 mL Opti-Fluor® organic cocktail in 7 mL glass vials, and the results were correlated with those from TopCount. Dose curves for the enzyme inhibitor and a control sample value obtained with TopCount were compared with those from the Packard Tri-Carb 2250CA.

A sandwich immunoradiometric assay (IRMA) for TSH was performed in a solvent resistant PicoPlate or in microplate strips (Dynex Technologies) coated with anti-TSH MAb as the first antibody. An ^{125}I-labeled anti-TSH MAb was used to quantitate TSH bound to the solid support by the first antibody. The PicoPlate was counted in the TopCount with 250 µL MicroScint-O, and individual wells of the microplate strip were counted in a Packard Cobra® gamma counter for comparison.

Results and Discussion

The average efficiencies and backgrounds obtained with TopCount with a small sample load are presented in Table 1. A small sample (10 µL) was used to provide the optimum cocktail performance. Counting efficiencies for ^{3}H are comparable to those which can be obtained by conventional liquid scintillation counting.

Radionuclide	Plate	Counting Efficiency %	Background (CPM)
^{3}H	96-well	45	19
^{3}H	24-well	47	50
^{14}C	96-well	88	35
^{14}C	24-well	92	62

Table 1.
^{3}H and ^{14}C performance in PicoPlates.

The CPM results for equal volumes of ^{3}H labeled samples in heptane counted with TopCount and a conventional LSC are compared in Figure 1. The data show that the TopCount correlates very well with the LSC (R^2= 0.99). The slope of the line indicates that for this sample load TopCount CPM is approximately 40% of the CPM obtained by conventional LSC. This lower efficiency is a result of the much higher sample load; only 250 µL of cocktail was used in TopCount versus 5 mL in the LSC.

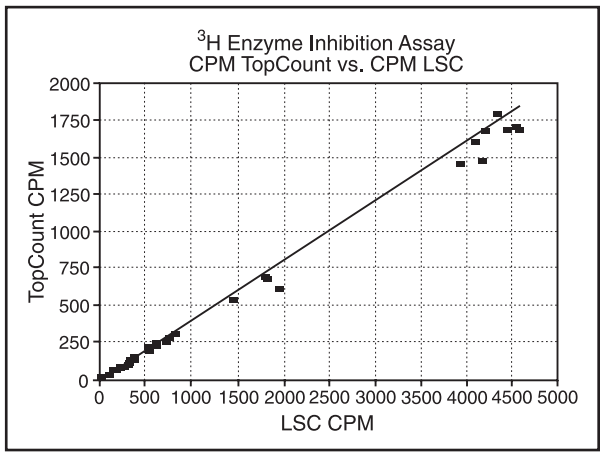

Figure 1.

A comparison of the enzyme inhibition curves obtained with the TopCount and the liquid scintillation counter is shown in Figure 2. The curves are essentially the same with both instruments. The results obtained with TopCount were independent of sample volume, despite sample loads of up to 50%.

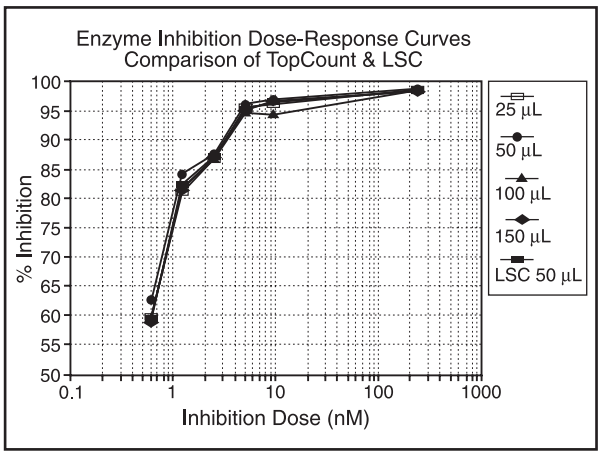

Figure 2.

Table 2 shows a comparison of the % inhibition values for a control sample obtained from TopCount with the value obtained by LSC. Assays 1-4 were counted with TopCount using four different sample volumes, and assay five was counted with the Tri-Carb 2250CA. These control values are additional confirmation that TopCount provides consistent results regardless of the sample load used in the assay.

Assay	Plate	Sample Size %	% Inhibition
1	96-well	25 µL	92.6
2	96-well	50 µL	92.5
3	96-well	100 µL	92.2
4	96-well	150 µL	92.5
5	LSC vials	50 µL	92.3

Table 2.
Comparison of inhibition values obtained from TopCount and LSC.

The results of an ^{125}I-labeled IRMA for TSH counted by liquid scintillation counting on TopCount and on a gamma counter are shown in Figure 3. The standard curves are essentially the same for the two methods of quantitation. Direct counting of immunoassay microplates with TopCount eliminates the need to transfer individual microplate wells to tubes for counting in a gamma counter. The counting efficiency for ^{125}I in TopCount, using MicroScint-O, is higher than 50%.

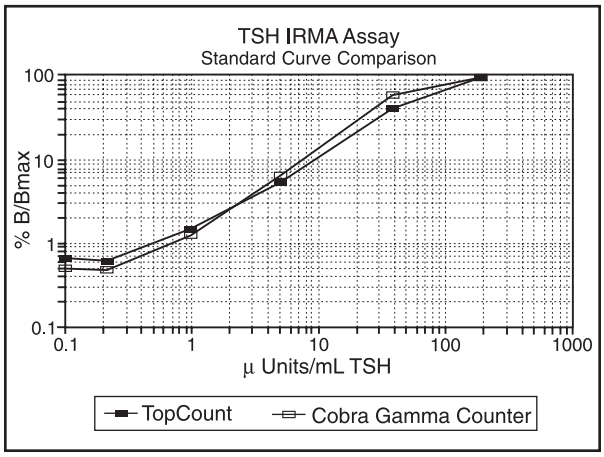

Figure 3.
TSH Immunoassay (IRMA) TopCount vs. Cobra.

Conclusions

The Packard TopCount Microplate Scintillation and Luminescence Counter, PicoPlates, and the specially formulated scintillation cocktail, MicroScint-O, provide results from samples in organic solvents or on solid supports equivalent to those obtained with a conventional liquid scintillation counter or gamma counter. Up to 50% sample loads of organic solvents may be used. With small sample loads, counting efficiencies are comparable to conventional LSC. For assays prepared in the microplate, TopCount provides quantitation in the same format. This results in increased throughput, less sample handling, and significant savings in reagent and waste disposal costs.

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