

TopCount *Topics*

TCA-002

Solid Scintillation Counting

Abstract

Solid scintillators are safe and convenient alternatives to conventional liquid scintillation cocktails. The Packard TopCount™ Microplate Scintillation Counter provides this technology with LumaPlates™, special microplates containing a solid scintillator. The radioisotope counting performance of LumaPlates in TopCount is comparable to or better than conventional scintillation counting. This paper presents TopCount performance results along with results from enzyme inhibition and cytotoxicity assays counted in LumaPlates. The assay results are compared to those obtained by liquid scintillation and gamma counting.

Introduction

Solid scintillation counting (SSC) is an attractive alternative to conventional liquid scintillation counting. With this method, a sample is deposited directly onto a solid scintillating material, dried, and counted in a scintillation counter. Small volumes of non-volatile, radioactively labeled samples in a volatile solvent can be quantitated. Samples from enzyme inhibition, cytotoxicity, immunoassay, receptor binding, and various metabolic studies can be counted with solid scintillators.

Solid scintillators have several advantages over liquid scintillators. They are not volatile, toxic, or flammable, and hence are safer to use. Waste disposal costs are reduced since the sample is dried onto the solid scintillating material and may be disposed of as solid waste. In some cases it is possible to recover dried samples for further processing, because they are not destroyed during the count-

ing process. For small volume, valuable samples, this can be the counting method of choice.

Although solid scintillation counting offers many advantages over conventional liquid scintillation counting (LSC), there has been no convenient way to utilize this technology for the variety of assays performed in the microplate format. Until recently, samples to be counted had to be individually pipetted or filtered onto the solid scintillator and placed in individual scintillation vials.

Packard Instrument Company has introduced the TopCount Microplate Scintillation Counter and LumaPlates, special microplates containing a thin layer of solid scintillator on the bottom of the wells. Samples prepared in either the 96- or 24-well format can be counted directly in that format. TopCount's multiple detectors count samples in a fraction of the time required by conventional scintillation counting. With TopCount, an assay prepared in the microplate format remains in that format for counting, thus helping to ensure positive identification by maintaining sample positions. Maintaining the microplate format also facilitates automated liquid sample handling. Thus, with TopCount, sample handling is reduced, throughput is increased, and positive sample identification is assured.

Solid scintillation counting using TopCount is evaluated here for radionuclide counting efficiency, DPM recovery with color quench, sample loading, and crosstalk. Where appropriate, correlations to conventional LSC are presented. In addition, we present results obtained by SSC with TopCount from an enzyme inhibition assay and a cytotoxicity assay.

Experimental Methods

Experiments were performed with the Packard TopCount Microplate Scintillation Counter with the VariPlate™ feature for counting in both 96- and 24-well formats. After samples were applied to the shallow well LumaPlates, they were air dried in a fume hood overnight. A heatlamp, hot air gun or centrifugal evaporators also may be used to dry the samples. After drying, the plates were sealed with Packard TopSeal™-P cover film using the MicroMate™ 496 Heat Sealer. All of the samples were counted for five minutes per well using a 0-256 channel window with the instrument protocol set for solid scintillator conditions. The isothermal counting chamber of the instrument was set at 19 °C. Samples were also counted in a Packard Tri-Carb 2250CA liquid scintillation analyzer or Packard COBRA gamma counter for correlation with TopCount.

Radionuclide Counting Efficiencies

Various radiolabeled compounds were used to assess the solid scintillation counting performance of TopCount:

| Radionuclide | Compound |
|---------------------|----------------------------------|
| ³ H | Thymidine |
| ³² P | ATP |
| ¹⁴ C | Thymidine |
| ⁵¹ Cr | Na ₂ CrO ₄ |
| ¹²⁵ I | IgG |

10 µL aliquots of the labeled compound in 100 mM sodium phosphate buffer containing 1 mg/mL BSA were dispensed in triplicate in either 24-well or 96-well LumaPlates. Additional 10 µL aliquots were prepared in triplicate for DPM determination in 10 mL of OptiFluor with a Packard Tri-Carb 2250CA liquid scintillation analyzer.

Color Quench Correction

Color quench and DPM recovery for colored samples was investigated by counting ¹⁴C in dye solutions in 96-well LumaPlates. Six concentrations of yellow dye were made with McCormick yellow food coloring concentrate to create a standard curve of efficiency versus quench. Four additional concentrations of dye were made to serve as test samples to check DPM recovery. 50 µL of each of the quenching solutions plus 10 µL of ¹⁴C-thymidine in 100 mL sodium phosphate buffer were dispensed in triplicate for each quench standard and sample. The ¹⁴C activity per well was approximately 93,000 DPM as determined by LSC.

Well-to-Well Crosstalk

To evaluate the extent of crosstalk between sample wells for ³²P, ⁵¹Cr, and ¹²⁵I, radioactivity was dispensed into a single 96-well LumaPlate well surrounded by two empty wells on each side (containing scintillator only). Results are reported as a percentage of the counts from the well containing radioactivity.

Sample Load

Varying volumes of 100 mM sodium phosphate buffer containing 1 mg/mL BSA and a constant amount of radioactivity (³H or ⁵¹Cr) were applied in triplicate to LumaPlate wells. For the 96-well plate a range of sample volumes from 10 to 60 µL was applied. For the 24-well plate the range was 10 to 360 µL. Counting efficiencies versus load of sample applied are presented for ³H and ⁵¹Cr.

Applied Performance - Enzyme Inhibition Assay

Samples from a ³H-labeled enzyme inhibition assay were counted with SSC on TopCount. The product of this assay had been extracted into heptane. Aliquots of 50 µL of each sample were dispensed in quadruplicate and counted with TopCount in the 96-well LumaPlate. The same size aliquots were counted in the Packard Tri-Carb 2250CA liquid scintillation analyzer. TopCount CPM versus LSC CPM correlations and dose response curves obtained with both instruments are presented.

Applied Performance-Cytotoxicity Assay

Samples of 50 μL from a ^{51}Cr release cytotoxicity assay were counted with SSC on TopCount and with a Packard COBRA gamma counter. The correlation of the TopCount results with those obtained with the COBRA are presented.

Results and Discussion

Radionuclide Efficiencies

Counting efficiency and background data for the 96- and 24-well LumaPlates are shown in Tables 1 and 2. LSC DPM served as our reference method for calculating TopCount efficiency. The efficiencies and backgrounds indicate that with the isotopes tested, TopCount performance is as good as or

| Radionuclide | % Counting Efficiency |
|----------------------|-----------------------|
| ^3H | 49 |
| ^{14}C | 85 |
| ^{32}P | 87 |
| ^{51}Cr | 24 |
| ^{125}I | 75 |
| Background = 8-9 CPM | |

Table 1.

TopCount 96-well counting efficiencies for various radionuclides.

| Radionuclide | % Counting Efficiency |
|------------------------|-----------------------|
| ^3H | 55 |
| ^{14}C | 95 |
| ^{32}P | 93 |
| ^{51}Cr | 48 |
| ^{125}I | 83 |
| Background = 19-20 CPM | |

Table 2.

TopCount 24-well counting efficiencies for various radionuclides.

better than that of LSC. In fact, in the 96-well format, sensitivity performance is better than typical LSC counting due to the low TopCount background (8-9 CPM compared to a typical LSC background of 15-25 CPM, depending on the counting region selected).

Color Quench Correction

As with liquid scintillation counting, the counting efficiency with solid scintillators is lower in the presence of color, because color absorbs some of the light produced by the scintillator. Increased color intensity results in increased color quenching and decreased CPM's. Figure 1 shows the ^{14}C counting efficiency versus tSIS (transformed Spectral Index of the Sample, a quench indicating parameter based on spectral energy distribution of the sample) for a set of standards containing increasing concentrations of color. The color quench correction curve can be used to recover ^{14}C DPM from the CPM data, as is shown in Figure 2. Excellent recoveries were obtained over a wide range of color quench. Also, the wavelength of the color quenching agent has very little effect on the recovery (see TopCount Topics TCA-004 for SPA counting).

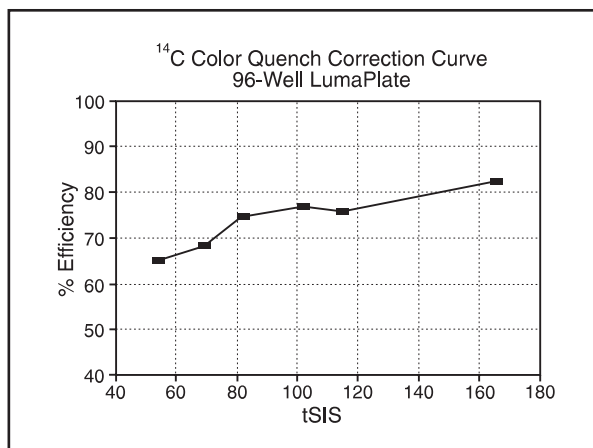


Figure 1.

TopCount ^{14}C color quench correction curve.

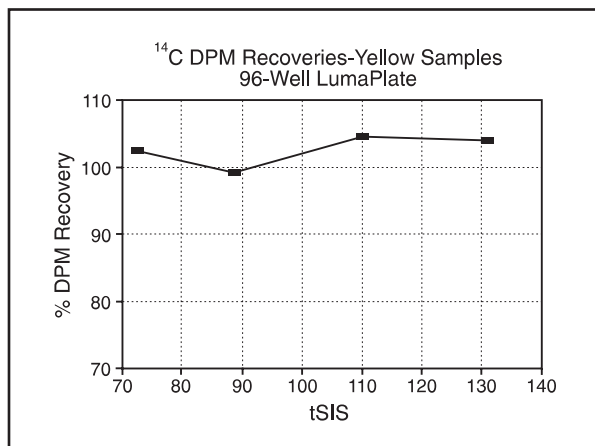


Figure 2.
TopCount ¹⁴C DPM recovery with LumaPlates.

Well-to-Well Crosstalk

Crosstalk from a well containing ³²P, ⁵¹Cr, or ¹²⁵I to surrounding wells is shown in Tables 3, 4, and 5, respectively. The results are presented as percentages of the activity measured from the central well containing the radioisotope. For the penetrating radiation from ³²P and ¹²⁵I, crosstalk of up to 0.6% and 0.4%, respectively, was observed (Tables 3 and 5). Two wells away the activity was at the background level. For ⁵¹Cr, the crosstalk into surrounding wells was less than 0.02% (Table 4). These results are for the 96-well LumaPlate. Crosstalk in the 24-well LumaPlate will be even less, due to the greater spacing between individual wells.

| | | | | |
|-------|-------|-----------------|-------|-------|
| 0.002 | 0.005 | 0.006 | 0.005 | 0.005 |
| 0.004 | 0.196 | 0.371 | 0.201 | 0.007 |
| 0.005 | 0.453 | ³² P | 0.575 | 0.010 |
| 0.004 | 0.183 | 0.457 | 0.247 | 0.008 |
| 0.002 | 0.004 | 0.006 | 0.006 | 0.004 |

Table 3.
% crosstalk of ³²P into surrounding LumaPlate wells.

| | | | | |
|-------|-------|------------------|-------|-------|
| 0.004 | 0.007 | 0.007 | 0.006 | 0.008 |
| 0.004 | 0.009 | 0.016 | 0.013 | 0.012 |
| 0.003 | 0.016 | ⁵¹ Cr | 0.019 | 0.014 |
| 0.004 | 0.007 | 0.016 | 0.008 | 0.010 |
| 0.003 | 0.004 | 0.002 | 0.006 | 0.012 |

Table 4.
% crosstalk of ⁵¹Cr into surrounding LumaPlate wells.

| | | | | |
|-------|-------|------------------|-------|-------|
| 0.009 | 0.029 | 0.035 | 0.035 | 0.011 |
| 0.025 | 0.153 | 0.350 | 0.117 | 0.032 |
| 0.010 | 0.372 | ¹²⁵ I | 0.346 | 0.012 |
| 0.020 | 0.125 | 0.340 | 0.141 | 0.031 |
| 0.009 | 0.026 | 0.042 | 0.042 | 0.016 |

Table 5.
% crosstalk of ¹²⁵I into surrounding LumaPlate wells.

Sample Load

The counting efficiencies for constant amounts of ³H and ⁵¹Cr in varying volumes of 100 mM sodium phosphate containing 1 mg/mL BSA are shown in Figures 3 and 4 for 96- and 24-well LumaPlates. These two radioisotopes were chosen because of their relatively low counting efficiencies in conventional LSC. There were no significant effects of the sample load on counting efficiency for the 96-well LumaPlate. There were small, but significant effects for both radioisotopes with the 24-well LumaPlate. The recommended maximum sample volume is 50-75 μL for a 96-well LumaPlate and 300 μL for a 24-well LumaPlate.

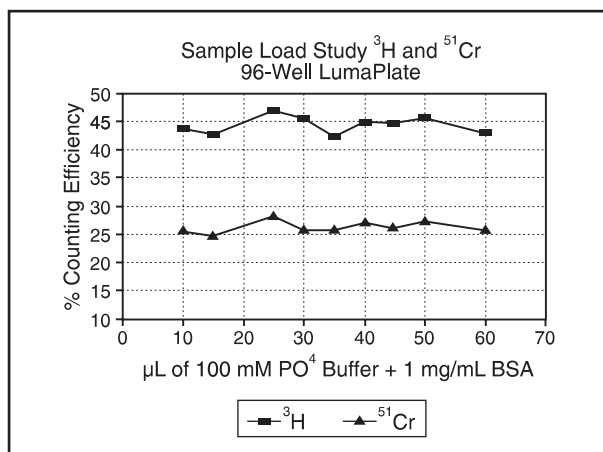


Figure 3.
³H and ⁵¹Cr counting efficiency vs. sample load, 96-well LumaPlate.

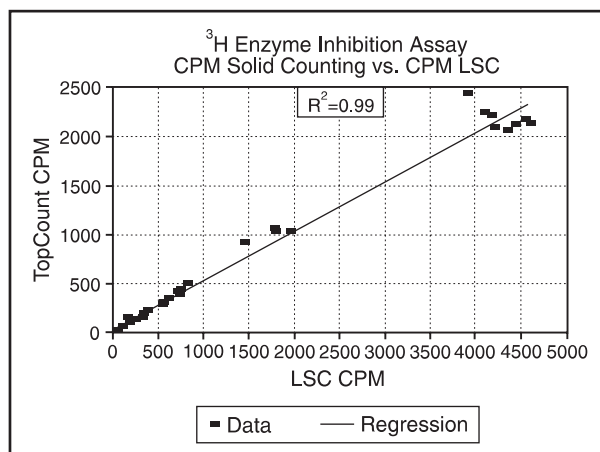


Figure 5.
 TopCount CPM vs. LSC CPM for ³H enzyme inhibition assay.

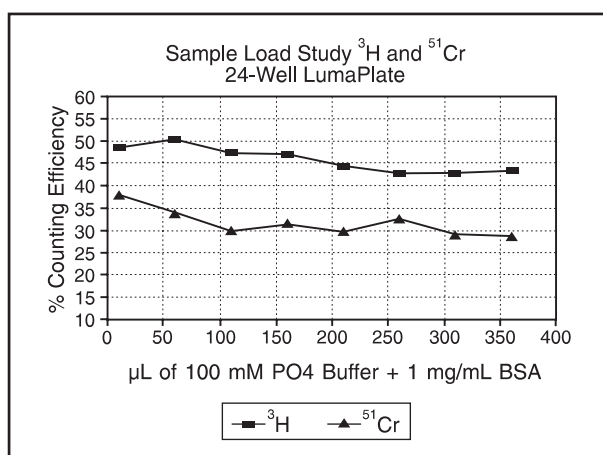


Figure 4.
³H and ⁵¹Cr counting efficiencies vs. sample load, 24-well LumaPlate.

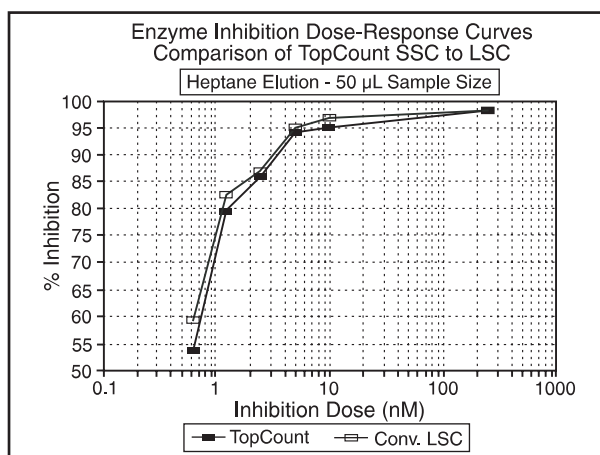


Figure 6.
 Enzyme inhibition curves, TopCount and LSC.

Enzyme Inhibition Assay

The ³H CPM results from an enzyme inhibition assay (Figure 5) show the excellent correlation of solid scintillation counting on TopCount and conventional LSC. The lower CPM by solid scintillation counting is probably due to proportional evaporation of the labeled compound during the sample drying steps. The dose response curves for the enzyme inhibition assay generated with TopCount and with LSC are equivalent (Figure 6).

Cytotoxicity Assay

There was a good correlation between the results from a ^{51}Cr cytotoxicity experiment obtained by solid scintillation counting on TopCount and those obtained on the COBRA gamma counter (Figure 7). Typically, the ^{51}Cr counting efficiency for LumaPlates is about five times that of a gamma counter. For the cytotoxicity samples shown in Figure 7, the ^{51}Cr counting efficiency is 2.5 times that of gamma counting due to the pink color of the samples which caused color quenching.

Conclusions

Because of the many advantages of the microplate format, it is used to perform many types of assays. TopCount provides a way to count samples in the microplate format in a fraction of the time required by conventional liquid scintillation counting. Throughput is increased, because up to twelve wells can be counted at once. With increasing concerns over the safety of liquid scintillation cocktails and their disposal, solid scintillation counting using LumaPlates is an attractive alternative to liquid counting. Counting efficiencies are comparable to those obtained with conventional liquid scintillation counting methods, and backgrounds are typically lower. For the detection of ^{51}Cr and ^{125}I , counting efficiencies are higher than can be obtained with conventional gamma counters. Assay costs are reduced, because less sample handling is required, scintillation vials and cocktails are not used, and no liquid waste is produced.

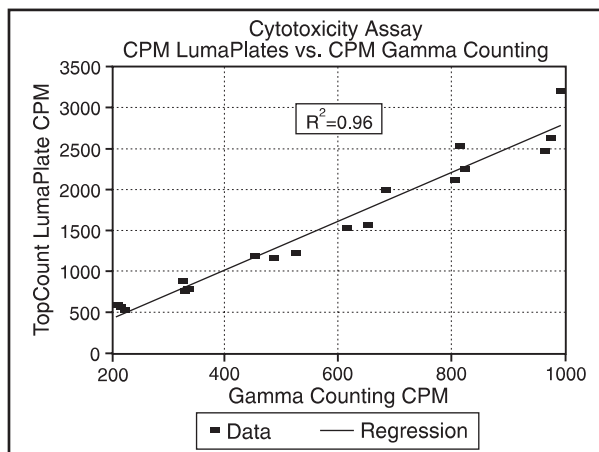


Figure 7.