TopCount *Topics*

TCA-024

Cerenkov Counting Performance on the TopCount™ Microplate Scintillation and Luminescence Counter

Abstract

The TopCount Microplate Scintillation and Luminescence Counter has been widely used in biopharmaceutical assays involving both radionuclide and luminescence measurement. A new feature allows the use of TopCount for measuring a high energy beta emitter such as ^{32}P by Cerenkov counting, a well-known measurement technique that has the main advantage of not requiring the addition of scintillation cocktail. Since cocktail is not required for measurement, there is little or no sample preparation, no chemical quenching, and the cost and time requirement to add cocktail is avoided. These advantages make Cerenkov counting an attractive alternative to liquid scintillation counting of ^{32}P in microplate based assays. Cerenkov counting performance in the TopCount is documented for a variety of sample matrices and assays. Typical TopCount efficiency, background, and crosstalk data are presented for both liquid and filter based assays. Quench correction for colored samples is also demonstrated.

Introduction

Cerenkov radiation occurs when charged particles traveling at or greater than the speed of light pass through a sample medium (usually liquid) where there is an exchange of energy from the charged particle to the molecules of the medium.[1] β energies in excess of 1 MeV are more desirable since the counting efficiencies are higher and closer to those observed by liquid scintillation counting. The main advantage to Cerenkov counting is the fact that scintillation cocktail is not required for measurement. The popularity of this technique is well documented for a variety of applications where higher energy beta emitters are used. For example, ^{32}P (E_{max} = 1710 keV) is used in molecular biology and enzyme activity assays because of the ease by which nucleotides and proteins can be labeled by phosphorylation reactions. Nucleotides labeled in this way can be used as probes in dot blot assays. The phosphorylation of proteins by kinases is an important cellular control mechanism. In addition, labeling of PCR products with ^{32}P allows easy quantitation of PCR reactions. All of these applications are easily done in a microplate or a microplate format dot blot apparatus. The sample is measured directly in its own medium with little or no preparation for counting.

In this report, the Cerenkov counting performance of the TopCount is documented for linearity, volume effects, efficiency, background and well-to-well crosstalk, as well as the applicability of this technique for liquid and filter/membrane counting. A protein kinase C assay was done to demonstrate the utility of this technique in a common application.

Experimental Methods

The Cerenkov counting mode was used on the TopCount with a factory set region of interest especially suited for highest sensitivity. Where appropriate, samples were also Cerenkov counted on the Packard Tri-Carb® 2500TR LSC for comparison to TopCount. The microplates were sealed with TopSeal™-A, a self-adhesive sealer. ^{32}P-ATP (Amersham #PB.10204) was the radioisotope used in all experiments. A stock solution containing 49,000 DPM/µL was used for all experiments.

Packard Instrument Company 800 Research Parkway, Meriden, CT 06450 Tel: 203-238-2351 1-800-323-1891
FAX: 203-639-2172 Web Site: http://www.packardinst.com

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PAN0077 1/96 Printed in U.S.A.

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Basic Performance

96- and 24-Well Microplates

Counting efficiency, crosstalk, and background data were acquired for counting in white 96- and 24-well OptiPlates™. The 96- and 24-well OptiPlate performance was evaluated using 200 µL and 1.0 mL, respectively, of water per well as the medium. Activity was added to the wells such that a spiked well was surrounded by eight neighboring blank wells containing only water [2] in order to evaluate well-to-well crosstalk. The crosstalk pattern was performed in triplicate for 96-well formats and in duplicate for the 24-well OptiPlate. All wells were counted for five minutes each using a predefined window for Cerenkov counting (channels 6-60).

Filter Plates

Two different 96-well filter plate formats were investigated, a UniFilter™ GF/B filtration plate[3] and a FlexiFilter™[4] containing a nylon membrane. FlexiFilter is a microplate format tray with an associated collimator designed to hold a membrane or filter of the user's choice. The 96-well UniFilter and FlexiFilter plates were counted wet. The same crosstalk pattern was used as described for OptiPlates.

Microcentrifuge Tube Counting

Counting performance in 0.4 and 1.5 mL microcentrifuge tubes containing varying amounts of water was assessed. Constant activity of 147,215 DPM was pipetted into a series of microfuge tubes. The 0.4 mL microcentrifuge tubes contained the following total volumes of water: 5, 10, 50, 100, 200, and 400 µL in duplicate. The 1.5 mL tubes contained 10, 50, 100, 500, 1000, and 1500 µL of water. Counting efficiency and backgrounds were determined on the TopCount by counting with the appropriate carrier microplates.

Volume Effect

The effect of different liquid sample volumes in 96- and 24-well OptiPlates was investigated. Triplicate wells were prepared with the following total volumes of water: 0 (dry), 10, 25, 50, 100, and 300 µL in a 96-well OptiPlate. Triplicate wells were prepared in a 24-well OptiPlate with the following water volumes: 0.5 mL, 1.0 mL, and 1.5 mL. All wells were counted for five minutes.

Performance Linearity

Count rate linearity was investigated in a 96-well OptiPlate by spiking wells in triplicate with dilutions of ^{32}P ranging from 1.3 million DPM to 40 DPM. The total volume of water per well was 150 µL. Each well was counted for five minutes.

Quench Correction

An experiment was performed to investigate the ability to correct for color quenching. A quench curve containing 151,000 DPM of ^{32}P per well was prepared in a 96-well OptiPlate using yellow food coloring as the quench agent as described in TopCount Topics, TCA-015.[5] The total volume of water per well was 200 µL.

Kinase Assays

A kinase assay was performed with the FlexiFilter to test the applicability of the Cerenkov counting technique with TopCount in a routinely performed filter based assay. A protein kinase C (PKC) assay kit (RPN.77, Amersham®) was used in the experiment. This assay measures the amount of PKC in a sample by the degree of phosphorylation of a specific peptide with [^{32}P]-ATP. The assay was performed essentially as described in TopCount Topics, TCA-018.[4] Serial dilutions of PKC (P-8289, Sigma®) were used to generate several levels of specific kinase activity ranging from 125 to 7.8 units of activity per well. Slight modifications were made in this assay compared to the one described in TCA-018. A 25 minute incubation instead of 15 minutes was performed; the stop reagent volume was reduced to 40 µL; and 15 µL instead of 10 µL of activity was spotted onto a precut sheet of phosphocellulose binding paper in a filtration manifold. After the assay was counted on TopCount, the individual sample spots were cut out from the filter and placed with 3 mL of water in glass 7 mL LSC vials and Cerenkov counted on a Packard Tri-Carb 2500TR liquid scintillation analyzer for comparison.

Results and Discussion

96- and 24-Well Microplates and Filter Plates

Table 1 shows the counting efficiency, energy crosstalk, and background CPM for a variety of microplate formats. Note that the crosstalk is not optical but is due to the highly penetrating radiation of ^{32}P beta particle interacting with neighboring wells. The data also indicate that the type of filter medium influences counting efficiency. The Cerenkov counting efficiencies on TopCount for UniFilter and FlexiFilter are about 50% of that obtained by Cerenkov counting on an LSC. Higher counting efficiencies on the TopCount can be achieved at the expense of a slightly higher background by adjusting the lower discriminator to a lower value.

Microcentrifuge Tube Counting

Average counting efficiency for 0.4 mL tubes was approximately 13% for all volumes of water added from 10 µL to 400 µL. Backgrounds are less than 300 CPM. A fairly constant counting efficiency over the sample volume range of 10 to 1500 µL was observed for the 1.5 mL tubes. The lowest observed was 13% (1500 µL) and the highest was 17% (500 µL volume). Background for the 6-60 chosen window will be less than 300 CPM. Higher counting efficiency can be obtained at the expense of background by raising the lower discriminator setting.

Volume Effect

Table 2 shows the TopCount Cerenkov counting efficiency performance as a function of volume of water sample in a 96- and a 24-well OptiPlate. The data shown are the mean of three replicates.

The data show that the volume of water influences the counting efficiency in a 96-well plate with maximum counting efficiency at the largest sample size. There does not seem to be a significant volume effect in the 24-well format.

Performance Linearity

The performance linearity data are shown in Figure 1 which is a plot of TopCount CPM versus DPM activity. The DPM was determined by counting the same activity on a Packard Tri-Carb 2500TR LSC. The figure shows that response is linear throughout the entire range of activities most likely encountered in experimental samples.

Microplate Type	% Crosstalk	Counting Efficiency %	Background CPM
96-well OptiPlate (200 uL sample)	0.4	25	20
24-well OptiPlate (1.0 mL sample)	0.3	25	93
UniFilter-96 GF/B (wet, 20 uL)	1.0	24	38
FlexiFilter nylon (wet, 20 uL)	2.0	25	38
0.4 mL microcentrifuge tube carrier	Not determined	13	<300
1.5 mL microcentrifuge tube carrier	Not determined	13-17	<300

Table 1.
Cerenkov counting performance in OptiPlates, UniFilter plates, FlexiFilter plates, and microcentrifuge tube carriers.

96-well OptiPlate		24-well OptiPlate	
Sample Size (uL)	Counting Efficiency (%)	Sample Size (uL)	Counting Efficiency (%)
Dry	10	Dry	14
10	12	500	22
25	14	1000	22
50	15	1500	23
100	18		
300	26		

Table 2.
96- and 24-well OptiPlate Cerenkov performance vs. volume of water sample.

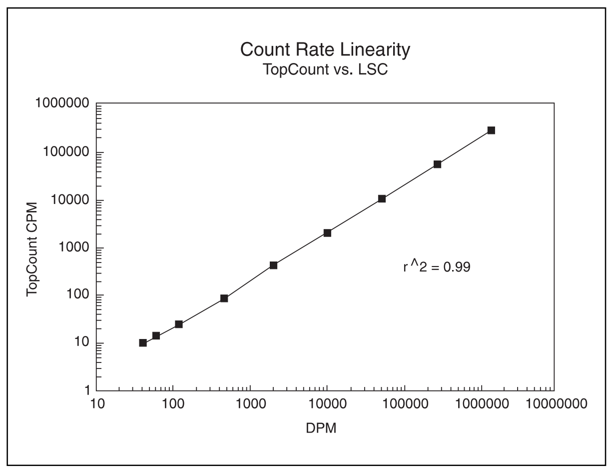

Figure 1.
Cerenkov counting response, CPM vs. DPM.

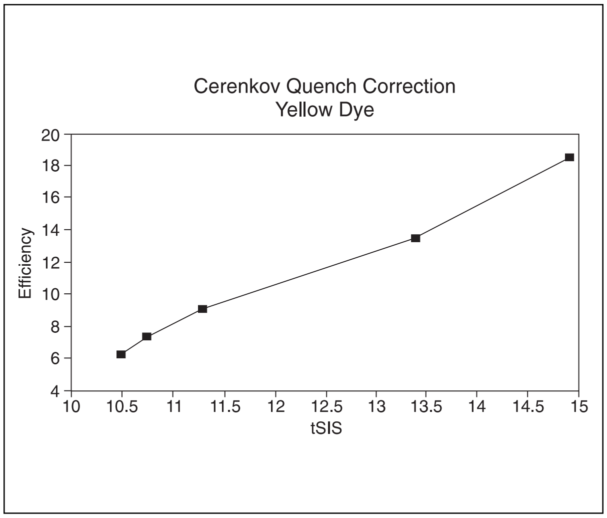

Figure 2.
^{32}P Cerenkov color quench curve.

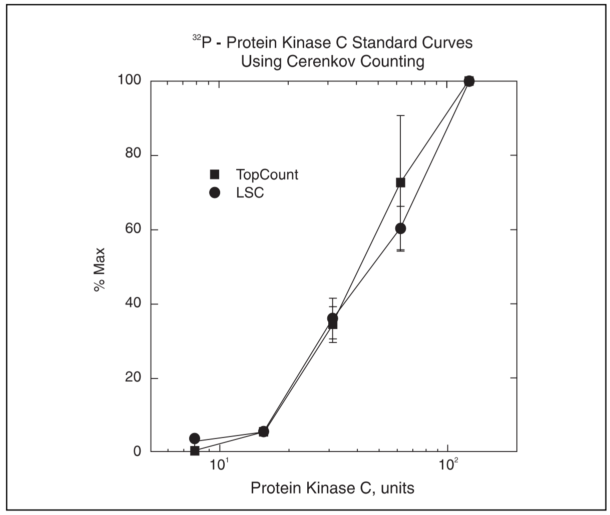

Figure 3.
Protein kinase C standard curves using Cerenkov counting.

Quench Correction

Figure 2 shows the ^{32}P quench curve prepared with yellow food coloring as the quench agent. Quench monitoring was performed with tSIS (a spectrum based quench indicating parameter exclusive to the TopCount). Figure 2 shows that tSIS is capable of accurately monitoring quenching due to color in samples that are Cerenkov counted.

Kinase Assay

Figure 3 shows a plot comparing the Cerenkov counting results from the kinase assay on both the TopCount and LSC. Data was plotted as % of the maximum binding on the respective instrument. As mentioned earlier, specific levels of kinase activity were measured on TopCount using FlexiFilter. The individual filter spots were removed from the FlexiFilter and Cerenkov counted in a Packard Tri-Carb 2500TR LSC for comparison. As seen in Figure 3, both the LSC and TopCount plots are nearly identical which indicates that equivalent final results will be obtained for the assay.

Conclusions

Cerenkov counting is an attractive alternative for various TopCount applications using ^{32}P.

An important advantage of Cerenkov counting on the TopCount is that it provides the ability to measure ^{32}P labels directly in the microplate without cocktail. This makes stripping and reprobing of ^{32}P labeled dot blots possible after counting. In addition, Cerenkov counting provides the ability to perform other molecular biology applications such as PCR product screening and labeling verification in a rapid and easy manner. Since cocktail addition is not required, throughput is increased because samples are counted directly.

For most microplate applications, the TopCount Cerenkov efficiency gives quantitative results and greatly reduces labor compared to Cerenkov counting in a conventional LSC.

References

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