TCA-031

^{33}P Counting Performance on the TopCount® and TopCount NXT™ Microplate Scintillation and Luminescence Counters

Abstract

The TopCount and TopCount NXT can be used to quantitate samples labeled with ^{33}P. Features such as RegionView and multichannel analysis can be used to optimize instrument set-up parameters for this radionuclide to produce high counting efficiencies, excellent pulse height distribution and dynamic range, as well as low background and crosstalk. ^{33}P is being used increasingly as an alternative to ^{32}P.

Introduction

^{33}P-labeled nucleotides have recently become commercially available and are gaining acceptance in many laboratories as alternatives to ^{32}P-labeled compounds. Advantages include a longer half-life and lower energy than ^{32}P. A comparison of the characteristics of each can be found in Table 1. These characteristics of ^{33}P result in safer handling in the laboratory as well as a longer useful life.

The TopCount is designed to count any radionuclide that emits beta particles, as well as the Compton electrons produced by many different gamma rays in scintillation cocktail. Analysis of the beta energy spectrum in the system's multichannel analyzer (MCA) allows the user to optimize counting conditions and detector set-up to achieve the best possible counting results. In addition, selectable energy ranges allow the user to optimize MCA gain so that spectral resolution is maximized for quench correction and/or dual label analysis.

The detector gain can be set up for low energy or high energy nuclides. Low energy nuclides are defined as those with endpoint energies equal to or less than ^{14}C (156 keV). Conversely, high energy nuclides generally have endpoint energies above that of ^{14}C. These guidelines are appropriate for most applications, but in some cases, it is desirable to empirically determine the optimum energy range. The specific energy distribution of a particular radionuclide may alter the apparent endpoint of the spectrum, and may result in an optimum setting different from the aforementioned guidelines. The RegionView and Endpoint Determination features in the TopCount are designed for this purpose and can be used to set both the energy range and counting regions for a particular nuclide or application.

A series of experiments was performed using [^{33}P]-ATP to determine the optimum operating parameters for a variety of common applications and detection strategies. Several types of microplates and filters/membranes were used. Counting efficiencies, quench parameters, and crosstalk were calculated for each case to demonstrate the relative performance for ^{33}P-labeled samples in each of the two energy ranges.

	^{33}P	^{32}P
Endpoint energy (keV)	249	1710
Half-life (days)	25.4	14.3

Table 1.
Characteristics of ^{33}P and ^{32}P.

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 Email: webmaster@packardinst.com

ISO 9001 SYSTEM CERTIFIED

M0976 4/98 Printed in U.S.A.

Materials and Methods

A set of microplates was chosen to represent a variety of applications and detection methods. [^{33}P]-ATP (catalog no. NEG602H, NEN™ Life Science Products) was diluted in 0.05 M PBS to an appropriate level. Two microliter aliquots of the diluted ATP were dispensed in triplicate into remotely located wells on each of the plates, as well as into three scintillation vials with which to determine absolute DPM on a Packard Tri-Carb® liquid scintillation analyzer. After drying the plate, the three wells containing ATP were injected with the appropriate type and volume of liquid scintillation cocktail. Eight of the nearest neighboring wells also received cocktail to check crosstalk. A summary of the plates and cocktails is listed in Table 2 below.

After sealing and mixing, a single sample from each plate was counted on the TopCount NXT to determine the spectrum endpoint in the “Low Energy” range. This was accomplished with the aid of the RegionView feature. Using this information, a Nuclide Library entry was established for the set of plates such that the primary counting region encompassed the entire radionuclide spectrum. Other detector set-up parameters were chosen appropriately for the type of plate being counted. All of the plates were then counted using these settings. The experiment was then repeated using the “High Energy” range setting. Counting efficiencies and crosstalk were determined as noted in Figure 1.

$$\% \text{ Counting Efficiency} = 100 * \frac{\text{Average TopCount CPM}}{\text{Average LSA DPM}}$$

$$\% \text{ Crosstalk} = 100 * \frac{(\text{Average of 8 neighbors - Background})}{(\text{Center Well - Background})}$$

Figure 1.

Results

Using RegionView, it was determined that all of the plates had spectrum endpoints between channels 198 and 256 in the “Low Energy” range. Therefore, a default counting region of 2.9-256 was established in the Nuclide Library for this range, and for the plates counted in that region. The lower discriminator setting of 2.9 channels was selected to eliminate virtually all of the low energy background. Table 3 summarizes the results.

These results demonstrate that high counting efficiency can be achieved for all sample types and crosstalk is very low in all cases. Spectral distribution is quite good, as shown by the high tSIS values (>150). This suggests that, if required, dual label separations with a low energy nuclide such as ^{3}H can be achieved by setting the Energy Range to “Low.” ^{3}H, which produces a tSIS of less than 20, can be separated from ^{33}P by choosing a discriminator of about 25.

Plate	Detection Technology	Cocktail	Vol (µL)
OptiPlate™	Liquid scintillation	MicroScint™-PS	200
FlashPlate®	Homogeneous binding assays	N/A	N/A
UniFilter® GF/C	Filtration assays	MicroScint-O	25
OmniFilter™, nylon membrane	Nucleic acid hybridizations	MicroScint-O	10
OmniFilter™, DE81 filter	Kinase assays	MicroScint-O	25
LumaPlate™	Solid scintillation	N/A	N/A

Table 2.
Test matrix for ^{33}P performance evaluation.

Plate	Scintillator	Efficiency Mode	Spectrum Endpoint (Channel)	Counting Region	CPM	% Efficiency	tSIS	% Crosstalk
OptiPlate	Liquid/Plastic	Normal	198	2.9-256	41758.7	71.2	158.9	0.07
FlashPlate			200	2.9-256	12805.7	21.8	154.4	0.04
UniFilter GF/C			250	2.9-256	48178.0	82.2	242.7	0.04
OmniFilter, nylon membrane			220	2.9-256	50731.7	86.5	176.2	0.02
OmniFilter, DE81 filter			202	2.9-256	38236.0	65.2	150.7	0.04
LumaPlate	Glass	High Sensitivity	256	0-256	48587.7	82.9	250.1	0.00

Table 3.
^{33}P counting results in low energy range.

It is important to note that the counting efficiency for the FlashPlate, while lower than that for the other sample types, is typical for a homogeneous assay format of this type. This is due to 2 π counting geometry and the use of plastic scintillators. These results are also typical of other proximity assay formats such as SPA™.

The results for the same samples, counted in the High Energy range, are summarized in Table 4. Here, all of the plates had spectrum endpoints between channels 26 and 45. Therefore, channels 0-50 were chosen as the counting region, and the plates counted as before. The results of this experiment demonstrate that counting efficiency is virtually the same as that found in Low Energy range. Crosstalk also remains extremely low.

Note, that in High Energy range, the nuclide spectrum is compressed into fewer MCA channels, as shown by the low tSIS values (<40). This demonstrates the effect of changing the Energy Range. It also suggests that dual label separations with a higher energy nuclide such as ^{32}P (which produces a tSIS greater than 150) can be done effectively in High Energy range by choosing a discriminator of approximately 50.

Plate	Scintillator	Efficiency Mode	Spectrum Endpoint (Channel)	Counting Region	CPM	% Efficiency	tSIS	% Crosstalk
OptiPlate	Liquid/Plastic	Normal	29	0-50	41678.3	71.1	20.7	0.07
FlashPlate			27	0-50	13493.0	23.0	20.1	0.04
UniFilter GF/C			45	0-50	47083.7	80.3	38.9	0.05
OmniFilter, nylon membrane			30	0-50	49214.3	83.9	22.7	0.02
OmniFilter, DE81 filter			26	0-50	37499.3	64.0	19.8	0.04
LumaPlate	Glass	High Sensitivity	235	0-256	48589.7	82.9	202.2	0.10

Table 4.
^{33}P counting results in high energy range.

Conclusions

^{33}P can easily be counted on the TopCount and TopCount NXT Microplate Scintillation and Luminescence Counters. High counting efficiency, excellent spectral distribution, and low crosstalk are maintained by the combination of time-resolved scintillation analysis, multichannel analysis and reflective optics. The use of RegionView, with its ability to optimize detector set-up parameters, allows the user to fine-tune the instrument for the particular application.

Although ^{33}P has an endpoint energy greater than 156 keV, its spectral distribution is such that better overall performance will be achieved if it is run as a "Low Energy" radionuclide. For most applications involving ^{33}P, use of the Low Energy range is recommended due to the greater spectral distribution and dynamic range of the tSIS parameter. This range allows the user to most accurately monitor changes in counting efficiency and correct for variable quenching conditions.