Abstract

Scintillation Proximity Assay (SPA) is a technique for performing binding assays without separation of bound and unbound radiotracer. Although sample preparation with SPA is simple, productivity has been limited by the available sample processing equipment. The TopCount® Microplate Scintillation and Luminescence Counter from Packard Instrument Company in conjunction with automated liquid handing equipment delivers dramatically higher throughput and automation of SPA procedures. The performance of the TopCount is compared with that of conventional liquid scintillation counters for representative SPA, RIA and receptor binding assays. The results presented illustrate that by counting in an isothermal counting chamber, the TopCount provides accurate micro-plate SPA results with enhanced throughput, resulting in faster results and decreased costs.

Introduction

Scintillation Proximity Assay (SPA) is a technology whereby binding reactions can be assayed without the washing or filtration procedures normally used to separate bound from free fractions. Assays are performed using radioactive labels that emit electrons with only a short range (about 10 um) in water. When bound close to a solid scintillator surface by the binding reaction they are able to transfer electron energy to the scintillator to produce photons detectable with a scintillation counter. Electrons emitted from labeled molecules not bound close to the surface dissipate their energy in the medium and are not detected. Thus the bound fraction is detected specifically without separation of the solution from the solid support.

The radioisotopes $^3$H and $^{125}$I, which are commonly used in ligand binding assays and radioimmunoassays (RIA), emit electrons with the low energies required for SPA. Thus many of these binding assays can be adapted to this new method, avoiding the usual filtration or washing procedures. SPA is attractive for these types of assays because they are often used as high volume screening procedures, typically in drug discovery, and high throughput and ease of automation are required for cost-effectiveness. Additional attractive features of SPA are that the progress of binding reactions can be monitored in time and that the bound fraction can be measured while in equilibrium with the free fraction.

Two types of SPA assay kits are commercially available. The first, primarily for RIA procedures, uses an inorganic glass scintillator, yttrium silicate. Other SPA kits, designed primarily for ligand binding assays, use plastic scintillator beads which have surface binding properties more suitable for peptide conjugation.

Existing scintillation counting technology has limited the throughput of SPA. With conventional scintillation counters, reagents must be dispensed into individual vials which must be capped and, after incubation, counted one sample at a time. Existing multiple-detector scintillation counters solve part of the throughput problem, but non-standard plate formats are not compatible with many liquid handling systems. Other microplate readers have severely limited well capacities. SPA samples may be
colored, and counting instrumentation must effectively correct for color quench so that all results are evaluated at a referenced quench level.

The TopCount is well suited for high throughput SPA analysis. TopCount uses standard format 96-well (8 X 12) rigid PicoPlates™, in which samples can be prepared by a number of commercially available robotic devices. These PicoPlate wells hold up to 400 µl and are highly reflective to maximize counting efficiency. The plates are opaque, so there is virtually no well-to-well crosstalk. A 24-well (4 X 6) PicoPlate is also available for applications which require sample volumes up to 1.5 ml. TopCount uses up to twelve detectors, resulting in greatly enhanced sample throughput with sensitivity comparable to conventional scintillation counting. Each detector employs a multichannel analyzer for accurate color quench correction. Kinetic measurements are possible with TopCount’s cycle counting capability.

We have performed scintillation proximity assays on TopCount using commercially available SPA kits which use either yttrium silicate or plastic scintillator beads. In this paper TopCount results for both ³H and ¹²⁵I labels are compared to those obtained with conventional scintillation counting. Color quench correction and well-to-well crosstalk on TopCount are evaluated.

**Experimental Methods and Results**

**General Procedures**

After incubation, microplates were assayed in the TopCount. The high sensitivity mode was used for assays using yttrium silicate beads. This mode results in extremely low background levels, yielding excellent assay sensitivity with yttrium silicate. The normal counting mode was used to maximize radionuclide counting efficiency with plastic scintillator beads which have different scintillation characteristics. Samples were counted with the count times and region settings recommended in the assay kit inserts. Conventional scintillation vials were counted similarly on a Tri-Carb® 2250CA or other discrete sample counter. All data sets were automatically analyzed using onboard applications software such as RiaSmart™ (Packard Instrument Company).

**SPA with Yttrium Silicate Beads**

To evaluate TopCount for use with yttrium silicate beads, assays were performed with SPA kits from Amersham® Corporation: a ³H 6-Keto-Prostaglandin-F1a SPA kit (Cat. No. TRK.952) and a ¹²⁵I cyclic AMP SPA kit (Cat. No. RPA.538). Reagent volumes were decreased by 25% to allow the use of the microplate, but otherwise the assays were performed according to the kit inserts. Standards and blanks were prepared in triplicate in wells of a PicoPlate and in 7mL plastic scintillation vials. The PicoPlates were covered with a heat-sealable protective film and were incubated on a microplate shaker at 1100 RPM for 16 hours. The LS vials were capped and incubated for the same period. Samples were counted in the appropriate instrument as described above.

Standard dose-response curves for the 6-Keto-Prostaglandin-F1a kit obtained with the TopCount and with a conventional scintillation counter are shown in Figure 1. The standard curves are similar. The raw data from the two instruments for this assay are plotted against each other in Figure 2. The high correlation coefficient and the slope approaching unity show that the TopCount results are indistinguishable from those produced on a conventional scintillation counter.

Similar results are summarized in Figures 3 and 4 for the cyclic AMP kit. Again, the standard curves from both instruments are very similar, and the linear regression illustrates excellent correlation.
RiaSmart, an RIA curve fitting and interpolation package available from Packard, was used to determine ED50, the estimated dose at 50% B/Bo. Using spline fits, the ED50 from TopCount was 20.9 fmol, and from the conventional counter it was 20.1 fmol. This further demonstrates that TopCount provides results that are virtually identical to those produced on conventional counting equipment.

SPA with Plastic Beads

To demonstrate the performance of TopCount for SPA on a plastic scintillator bead, an **125**I Angiotensin assay kit (Amersham International plc) was processed according to the recommended procedures. Reagents were dispensed into wells of a Dynex® MicroFLUOR® microplate (white) for counting with TopCount or into 1.5 mL microcentrifuge tubes for counting in a conventional scintillation counter. Total sample volumes were 150 µL. After a three hour incubation period, samples were counted as described previously.

The similar dose-response curves and excellent correlation between instruments for the Angiotensin kit (Figures 5 and 6) again show that TopCount provides results comparable to those from conventional counting equipment. With these plastic beads, the counting efficiency is somewhat lower with TopCount than with LSC. This is a consequence of the light emission characteristics of the scintillators in these beads. The ability to assay up to 12 samples simultaneously compensates for the lower counting efficiency, and provides enhanced sample throughput.

Color Quench Correction

Color quenching in scintillation counting can be overcome by establishing a quench correction curve...
using a series of progressively quenched, constant activity standards. To demonstrate correction for colored samples in TopCount, SPA beads coupled with a \(^3\)H labeled peptide (Amersham International plc) were counted with various levels of color quench. Three sets of twelve samples (100 \(\mu\)L) were dispensed into microplate wells, and increasing amounts of red, yellow or blue dye solutions were added to successive wells of respective sets. The microplate was covered, thoroughly mixed, and counted in TopCount.

The quench correction curves for the three dyes are superimposed in Figure 7. This indicates that a spectrum-based quench correction such as tSIS can correct colored samples to a consistently low reference quench level using a single color quench correction curve independent of color hue.

**Well-to-well Crosstalk**

Microplate scintillation counting places radiolabeled samples and detectors close to each other. This could cause significant well-to-well crosstalk if photons produced in one well were detected in neighboring wells. To evaluate the extent of crosstalk, the \(^3\)H labeled SPA beads were dispensed into selected wells on a PicoPlate. Unlabeled SPA beads were added to the surrounding wells to simulate assay blanks placed next to high activity samples. The plate was covered and counted in TopCount.

Results from wells neighboring the radioactive sample were indistinguishable from background (data not shown). Samples with widely varying activities can be placed next to each other without loss of accuracy.

**Conclusions**

Scintillation Proximity Assay is a method for performing competitive binding assays such as immunoassays and receptor binding assays with a minimum of sample handling. SPA results obtained with the TopCount Microplate Scintillation and Luminescence Counter are comparable to those obtained with a discrete sample scintillation counter, and with as many as 12 detectors, TopCount isothermal counting chamber can enhance counting throughput dramatically. TopCount permits counting in conventional microplates, facilitating the use of commercially available liquid handling systems. True hands-off processing can be achieved with Tandem Processing from Packard, which allows unattended data reduction from raw counts to interpolated dose or ligand concentration values using an on-board IBM® compatible computer. Significant reductions in labor, turnaround time, and cost can thus be obtained.

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Abstract
The TopCount™ Microplate Scintillation Counter quantitates radioisotopes by liquid scintillation counting in standard microplate formats. Assays that previously had to be transferred from microplates to vials or to non-standard formats for counting can now be quantitated directly in microplates. TopCount can count up to 12 samples simultaneously with performance comparable to that of discrete sample liquid scintillation counters. Effects of sample quench can be corrected with quench indicating parameters based on the sample spectrum (tSIS) or, when using the 4 X 6 format, external standard spectrum (tSIE). With multiple detectors and the microplate format, TopCount offers greater throughput, decreased cocktail consumption, more efficient sample handling, and more secure sample identification. Samples from a cytotoxicity assay and an enzyme inhibition assay were counted on TopCount, and the results were compared to those obtained with conventional liquid scintillation and gamma counting.

Introduction
Biological assays are frequently performed in 96-well or 24-well microplates, and the ability to quantitate radioactivity directly in microplate wells by liquid scintillation counting is very desirable. With the Packard TopCount Microplate Scintillation Counter, liquid samples can be counted directly in solvent resistant microplates (PicoPlates™) with a choice of cocktail formulations that will accommodate a wide variety of samples. For aqueous samples, two TopCount cocktails, MicroScint™-20 and MicroScint™-40, have been specially formulated to provide optimal performance with up to about 20% and 40-50% of water, respectively. These cocktails have the desirable features of an environmentally benign solvent, excellent sample holding capacity, and quench resistance.

TopCount simultaneously counts up to 12 samples in the 96-well format or six samples in the 24-well format. By maintaining the microplate format for quantitation, less sample handling is required, pipetting errors are minimized, and liquid handling can be facilitated with multiple pipetting devices or standard automatic liquid handling systems. TopCount uses less scintillation cocktail than conventional systems and thus, minimizes waste disposal costs.

TopCount is evaluated here for counting efficiency, quench correction and DPM recovery for single and dual radiolabel counting, cocktail quench resistance, and crosstalk between wells. In addition, samples from a cytotoxicity test and an enzyme inhibition assay were counted with TopCount, and with gamma counting or conventional liquid scintillation counting (LSC) for comparison.

Experimental Methods
Counting Procedure
All samples were counted on a TopCount Microplate Scintillation Counter with the VariPlate™ feature, which allows counting in either the 24- or 96-well format. Samples were added to scintillation cocktail
in the wells of a 24-well or 96-well PicoPlate with a multiple-tip pipettor. The plates were sealed with TopSeal™-P, a solvent resistant protective cover film, using a Packard MicroMate™ 496 Microplate Heat Sealer. After shaking the plates on a microplate orbital shaker for 15 minutes, each sample was counted on TopCount for five minutes. Samples were also counted on a Packard Tri-Carb 2250CA liquid scintillation analyzer (LSC) and a Packard COBRA gamma counter for comparison.

Radionuclide Counting Efficiencies
The counting efficiency of TopCount for a variety of radionuclides was assessed by counting the following compounds:

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<tr>
<th>Radionuclide</th>
<th>Compound</th>
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<tr>
<td>³H</td>
<td>Thymidine</td>
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<tr>
<td>³²P</td>
<td>ATP</td>
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<tr>
<td>¹⁴C</td>
<td>Thymidine</td>
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<tr>
<td>⁵¹Cr</td>
<td>Na₂CrO₄₂⁻</td>
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<tr>
<td>¹²⁵I</td>
<td>IgG</td>
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Aliquots of 10 µL were counted in triplicate in both 96-well PicoPlates and 24-well PicoPlates containing 250 µL and 1.0 mL MicroScint-20, respectively. The same number of wells containing only cocktail were used for background determinations.

Chemical Quench Correction
Chemical quench correction for ¹⁴C was studied in 96-well PicoPlates using tSIS, the transformed Spectral Index of the Sample, as the quench indicating parameter. With 24-well plates an external standard can be used to determine the quench level of a sample, and tSIE, the transformed Spectral Index of the External standard, was used to indicate quench. Quench correction was evaluated in 24-well plates for ³H and ³H/¹⁴C dual label DPM recovery.

Color Quench Correction
To study DPM determination with colored samples, a ¹⁴C color quench curve using tSIS was prepared in the 96-well format. Various concentrations of McCormick yellow food coloring in MicroScint-20 scintillation cocktail were prepared, and ¹⁴C thymidine was added to triplicate wells containing 250 µL of the color quenched cocktail and counted on TopCount. A similar set of color quenched samples was prepared as unknowns for DPM determination.

Sample Load and Quench Resistance
The effect of sample load on counting efficiency in both MicroScint-20 and MicroScint-40 cocktails was investigated by counting a constant amount of ³H thymidine in the presence of increasing volumes of water or aqueous solutions of 0.15M NaCl, 5% phosphoric acid or 5% trichloroacetic acid (TCA). The total volume of sample and cocktail was 300 µL for the 96-well PicoPlate and 1.0 mL for the 24-well plate.

Well-to-Well Crosstalk
Crosstalk to adjacent wells for ³²P, ¹⁴C, ⁵¹Cr and ¹²⁵I was investigated by adding the radionuclide to only the central well of nine wells containing 250 µL of MicroScint-20. The crosstalk is reported as a percentage of the activity in the central well.

Applied Performance
Duplicate aliquots of 50 µL from ⁵¹Cr cytotoxicity assay samples were counted in a 96-well PicoPlate