Scintillation Proximity Assay on the TopCount Microplate Scintillation Counter

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 microplate 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 µm) 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 ^{3}H and ^{125}I labels are compared to those obtained with conventional scintillation counting. Color quench correction and well-to-well crosstalk on TopCount are evaluated.

800 Research Parkway Meriden, CT 06450 Tel: 203-639-2598 1-800-323-1891 Fax: 203-639-2172
Web site: http://www.packardinstrument.com E-mail: webmaster@packardinstrument.com

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 ^{3}H 6-Keto-Prostagland in-F1a SPA kit (Cat. No. TRK.952) and a ^{125}I 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 7 mL 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.

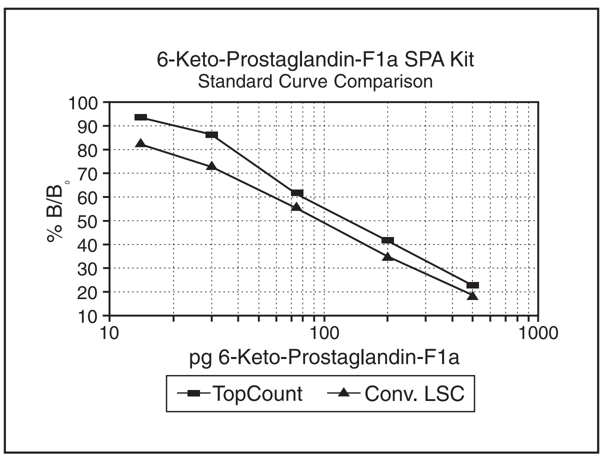

Figure 1.

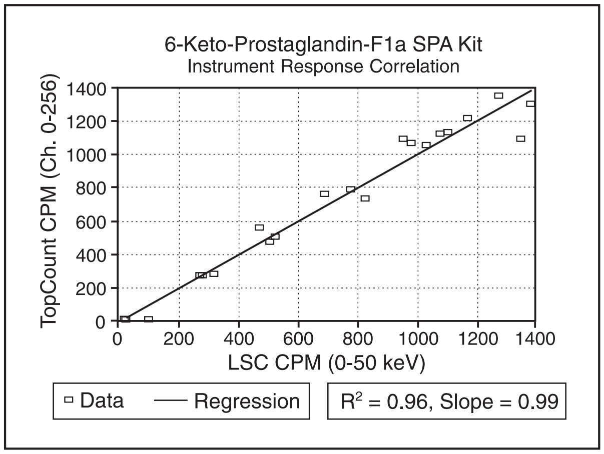

Figure 2.

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.

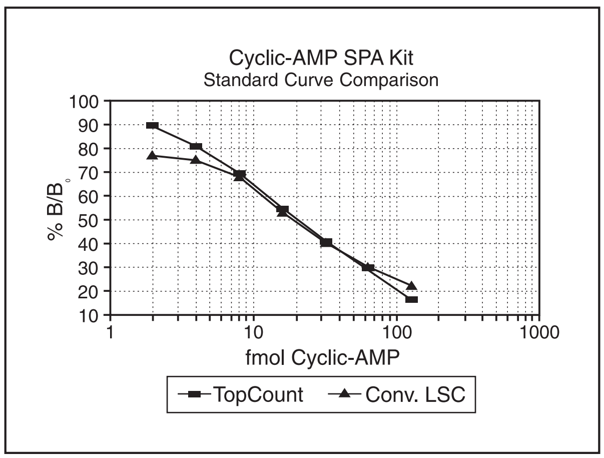

Figure 3.

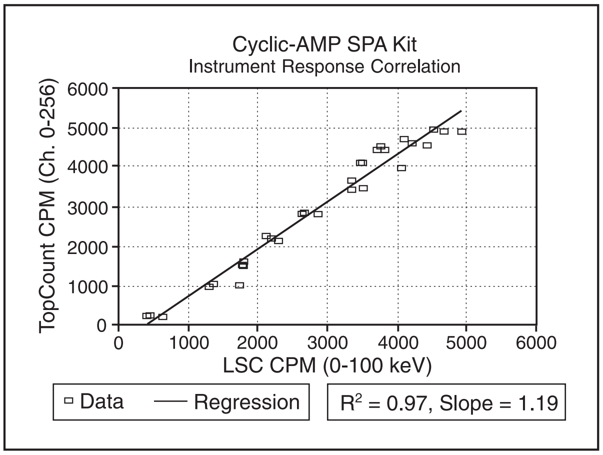

Figure 4.

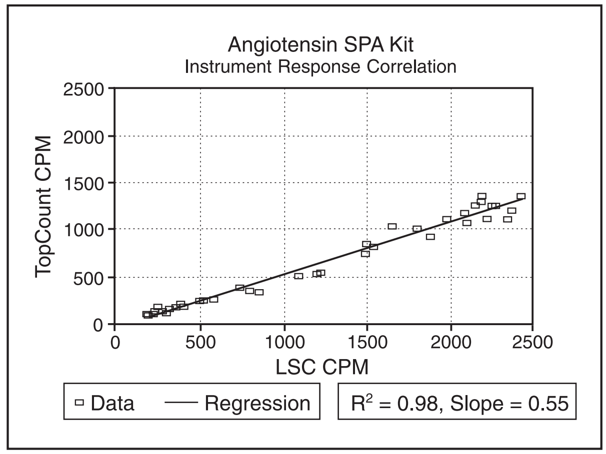

Figure 6.

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.

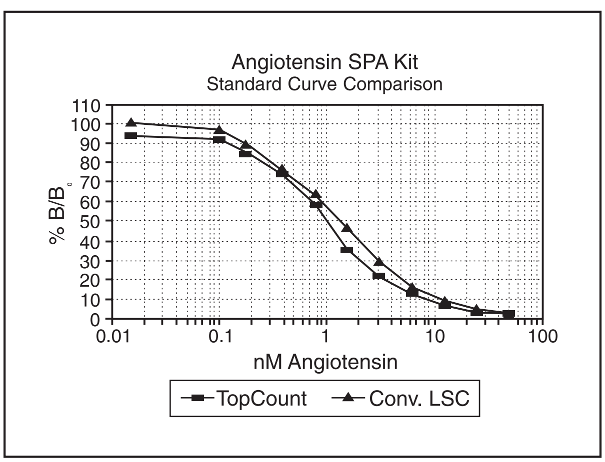

Figure 5.

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 µL) 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.

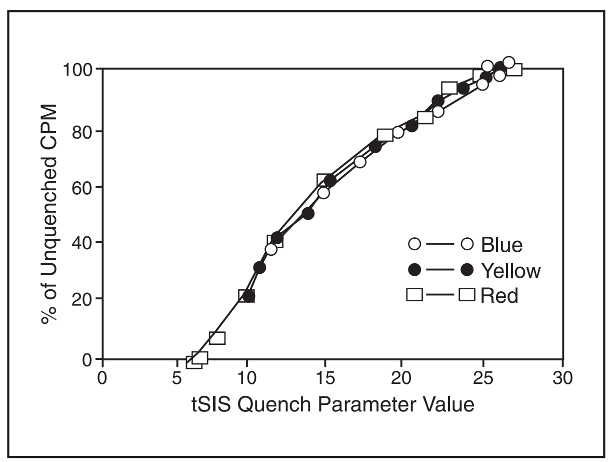

Figure 7.
SPA color quench correction % of unquenched CPM vs. QIP.

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.

References

1. Amersham is a registered trademark of the Amersham Corporation.
2. Dynex and MicroFLUOR are registered trademarks of Dynex Technologies.

Reprint Notes:

The TopCount instrument is a microplate scintillation and luminescence counter that is currently capable of counting samples in 24-, 96- and 384-well formats. (M0639 10/97)

The TopCount system currently counts at a user-selectable constant temperature 15 to 30 °C, when operated at ambient temperatures of 15 to 35 °C.

Correction: First paragraph in Introduction section. Range should be expressed as 10 µm.

M4163 (M0639-01) 5/01 Printed in U.S.A.