

# TopCount *Topics*

TCA-011

## Direct Counting of Millipore® MultiScreen® Filtration Plates.

### Abstract

The TopCount Microplate Scintillation and Luminescence Counter can be used to count samples prepared in MultiScreen microplates. These plates are designed so that all assay steps are performed in a single microplate. However, conventional approaches still require the transfer of filter disks into discrete liquid scintillation (LS) vials or gamma tubes for quantitation. TopCount offers the ability to count labeled samples in MultiScreen filtration plates directly, thereby minimizing handling and consumables costs and increasing sample throughput. This paper describes several studies comparing direct counting in TopCount to conventional methods.

### Introduction

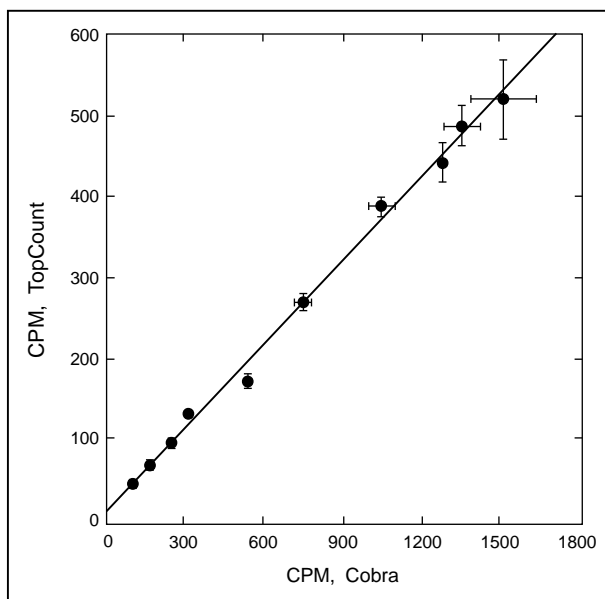
The MultiScreen Assay System (Millipore Corporation) is a microplate filtration system suited for screening procedures that require separation of bound from free radiolabel. Assays which have been performed in the MultiScreen system include cell-based receptor binding and cell proliferation studies. A variety of other applications can be performed with MultiScreen. A number of publications from Millipore Corporation, entitled "MultiScreen Methods", describe various applications in detail.

The MultiScreen system consists of filter-bottom microplates made with one of several types of membrane filters. The membrane-bottom wells can hold aqueous buffers and assay media within the plate until separation is carried out on a vacuum filtration manifold. After removing the underdrain, the 96 individual filters are

typically punched into LS vials or gamma tubes using a punch device supplied by Millipore. The MultiScreen system permits significant increases in sample preparation speed, since pipetting and incubation can be done with automated microplate equipment. However, overall throughput is hampered by the need to punch samples into discrete counting vials. Large numbers of vials, significant amounts of LS cocktail, and much labor are required.

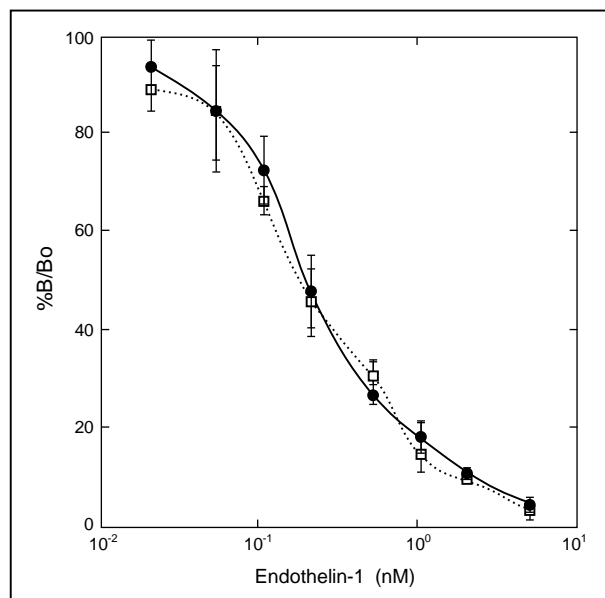
The TopCount Microplate Scintillation Counter can be used to dramatically reduce costs and throughput when using the MultiScreen system. MultiScreen filtration plates manufactured by Millipore Corporation are available in a white, solvent-resistant plastic that is designed to prevent optical crosstalk between wells and to contain liquid cocktails. These plates provide optimum performance in the TopCount. After conducting set-up, incubation, and separation steps directly in the plate, it is snapped into a reflective adapter available from Packard Instrument Company. This permits the addition of volumes of LS cocktail sufficient to maximize counting efficiency for low energy isotopes such as  $^3\text{H}$  and  $^{125}\text{I}$ . LS cocktail is easily added to each well using automated equipment. This eliminates tedious punching and vial filling/capping procedures, and permits the use of the microplate stacker and bar code sample identification systems on TopCount. With up to 12 simultaneously counting detectors, TopCount is capable of significantly increasing counting throughput.

Presented here are the results of several experiments which demonstrate the performance of the MultiScreen system on the TopCount Mi-



**Figure 1.**

Correlation of raw counting results from [<sup>125</sup>I]-Endothelin-1 assays performed in MultiScreen filtration plates by direct counting on TopCount and by punching of filter disks into vials for gamma counting. The data are fit with a linear regression line,  $R^2 = 0.99$ .



**Figure 2.**

Competition curves showing the inhibition of binding of [<sup>125</sup>I]-Endothelin-1 by unlabeled Endothelin-1. Parallel experiments were performed on TopCount (solid circles and solid line) and on a Cobra gamma counter (open squares and dashed line).

croplate Scintillation Counter.

## Experimental

### Cellular Receptor Binding.

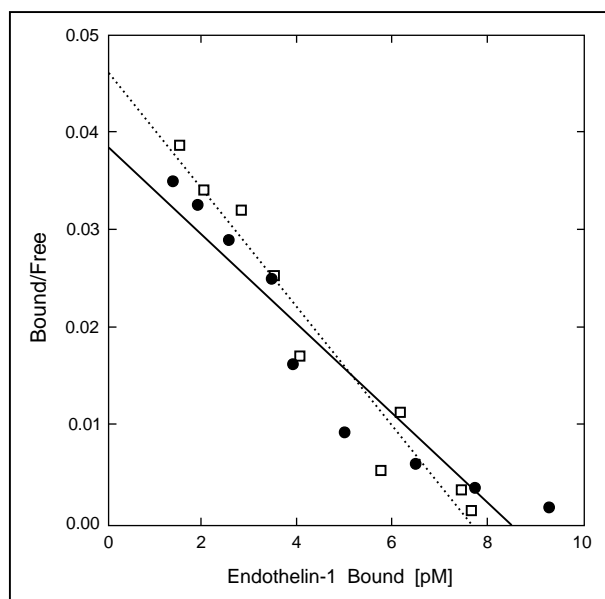
Competitive binding assays, typically done using cell harvesters and glass fiber filter media, are designed to assess the competition between a test compound and a labeled ligand for a cell surface receptor. A number of standard binding assays have been commercialized in kit form. For this experiment, we chose the Endothelin-1 kit (<sup>125</sup>I) available from DuPont-NEN<sup>®</sup> Research Products, Boston, Massachusetts (Cat. No. NED-009).

We used the white MultiScreen-HV plate (Millipore<sup>®</sup> Cat. No. SA2M060E2), which contains a Durapore PVDF 0.45  $\mu$ m membrane. A standard competition curve was prepared, following the instructions provided with the reagents. After incubation, the ligand-receptor complex was separated from free radioligand on the MultiScreen vacuum manifold (Millipore Cat. No. MAVM09601). The plate was then dried and snapped into the adapter (Packard # PPN 6005178). 25  $\mu$ L of MicroScint-O, a lipophilic LS cocktail designed for TopCount, was dispensed into each well using a multichannel pipet. After covering with TopSeal-S protective sealing film, the plate was loaded and counted directly in TopCount.

A second plate was set up in parallel. The individual membranes on this plate were punched, using the MultiScreen Punch Kit (Millipore Cat. No. MAPK8960C), into 12 X 75 mm gamma tubes and counted on a Packard Instrument Company Cobra<sup>®</sup> gamma counter. Figure 1 shows the correlation of counting data for the plates counted on the two instruments, and Figure 2 shows the resulting standard competition curves.

Total ligand tubes were also prepared and counted in the Cobra. After conversion of all counting results to DPM, a Scatchard analysis was performed (Figure 3). The  $K_d$  and  $B_{max}$  determined with the two instruments are compared to reference values in Table 1.

These results show that cellular receptor binding assays can be performed in the MultiScreen filtration plate and counted directly in TopCount. Counting efficiency averaged 31.4%, with



**Figure 3.**

Scatchard analysis for Endothelin binding performed with TopCount (solid circles and solid line) and with Cobra (open squares and dashed line).

crosstalk not exceeding 0.25%. Final results are equivalent to those obtained by gamma counting.

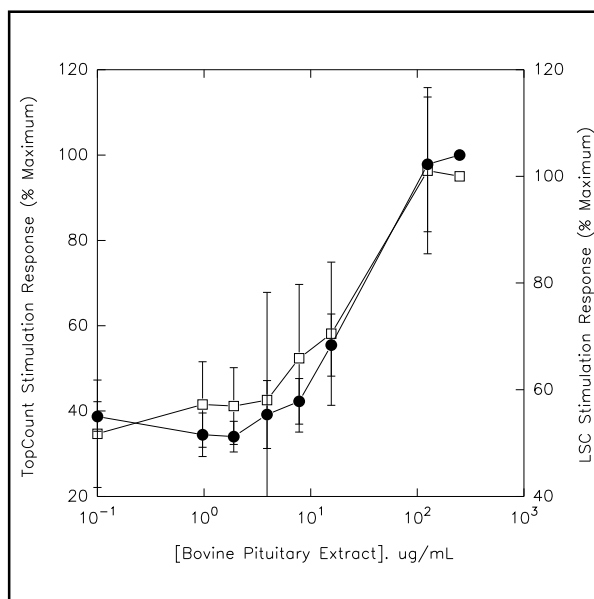
#### Cell Proliferation Assay.

For this assay, we also chose the MultiScreen HV plate (Millipore® Cat. No. SA2M060E2). The plate was sterilized and treated with 25% Rat Tail Collagen, Type 1 (Collaborative Biomedical Products®, Bedford, Massachusetts, #40236) to improve cell growth. 3T3 cells (ATCC, Rockville, Maryland) were cultured to  $10^4$  cells per well and stimulated with Bovine Pituitary Extract (Collaborative Biomedical Products, #40123) at levels ranging from 0 to 250 ug/mL for 18 hours. The cells were then pulsed with  $^3\text{H}$ -thymidine for three to four hours

	$K_d$ (pM)	$B_{max}$ (pM)
TopCount	222	8.5
Cobra Gamma Counter	169	7.7
Reference Values	168	19.2

**Table 1.**

$K_d$  and  $B_{max}$  values for Endothelin. Reference values were provided with the NENQuest kit.



**Figure 4.**

Cell proliferation in response to bovine pituitary extract determined in parallel experiments on TopCount (solid circles and solid line) and by LS counting (open squares and dashed line).

prior to washing on the MultiScreen vacuum manifold. Two plates were prepared. The first was processed by punching the individual membrane disks into LS vials, solubilizing with 0.42% bleach, and mixing with 5 mL LS cocktail (Optifluor, Packard Instrument Company). The second plate was processed by adding 20 $\mu\text{L}$  MicroScint-O to each well using a semi-automatic multichannel pipet and counting on TopCount.

Stimulation curves obtained with the TopCount and with conventional LSC are shown in Figure 4. Both methods yield the same results.

MultiScreen filtration Plate	Radionuclide	Counting Efficiency
HV	$^3\text{H}$	7-21%*
	$^{35}\text{S}$	75%
	$^{32}\text{P}$	75%
HA	$^{14}\text{C}$	85%
	$^{125}\text{I}$	46%

**Table 2.**

Typical counting efficiencies obtained for MultiScreen filtration plates on TopCount, optimized counting conditions. \*Efficiency depends on assay and counting conditions.

### **Other Assays.**

A variety of other assays such as enzyme inhibition and kinase assays have been performed with the MultiScreen filtration plate on TopCount. Counting efficiencies for the radionuclides used in these assays are summarized in Table 2. For weak beta emitters such as  $^3\text{H}$ ,  $^{35}\text{S}$ , and  $^{14}\text{C}$ , crosstalk was negligible. For gamma emitters ( $^{125}\text{I}$ ) and higher energy beta emitters ( $^{32}\text{P}$ ), crosstalk was less than 0.5%.

### **Conclusions**

The Millipore MultiScreen Assay system is designed for a variety of microplate assays requiring in plate incubations and filtration. A significant limitation of the system is the need to individually process the 96 samples in a plate for quantitation. The TopCount Microplate Scintillation Counter can facilitate the processing of MultiScreen filtration plates by allowing the user to process all 96 samples as a single unit. Crosstalk due to physical migration of radiolabel is not possible due to the discrete filter disks in the MultiScreen filtration plate. TopCount's reflective optics and adapter plate prevent optical crosstalk and provide accurate, quantitative

results. Assay results obtained using TopCount will be comparable to those obtained on conventional counting equipment, cocktail usage can be reduced by a factor of 100, and considerable savings in labor, time, and costs can be achieved.

### **Acknowledgments**

We would like to thank A. M. Pitt, Millipore Corporation, Bedford, Massachusetts, for cell culture and sample preparation for the cell proliferation assay.

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