

## BIOLOGICAL APPLICATIONS OF MICROPLATE SCINTILLATION COUNTING

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**ABSTRACT.** The microplate format has become increasingly popular in biological experimentation due to its small size, easy sample handling, increased sample preparation throughput and small sample volume. The need to perform large numbers of assays has sparked interest in high-throughput, microplate-compatible radioisotope counters. Many of these biological experiments are performed on glass fiber filters or synthetic membranes. These media may be used to separate specific components of an assay mixture for counting, or target substances may be immobilized for analysis with radiolabeled or luminescent probes. The diversity of assays requires a number of different filter types and sizes. We have developed a microplate scintillation and luminescence counter and associated accessories with which to carry out many of these procedures in several popular formats. We describe here experiments that demonstrate the feasibility of using microplate-based instrumentation for several classes of important biological assays.

### INTRODUCTION

Modern techniques used in biological and pharmaceutical research involve the assay of large numbers of samples. Various biological agents and drug candidates are routinely evaluated by assessing their ability to affect binding to neurotransmitter and cell-surface receptors, enzymatic activity, cell growth and immune function. Compounds are run through a large panel of tests to evaluate their potential as therapeutic agents. We conduct many assays by collecting bound material on glass fiber or synthetic membranes, using a cell harvester or filtration manifold and washing through the unbound tracer. The filter must then be assayed in a liquid scintillation counter (LSC) or gamma counter to assess the action of the test compound. In recent years, the microplate format has become popular for these types of tests. Microplates provide a convenient and efficient format, in which replicates, blanks and controls can be arrayed in a convenient matrix for pipetting with multichannel pipettors or with sample-handling robots. Conducting experiments in a microplate reduces sample handling and radioactive waste, because entire assays can often be performed in a single plate. Until very recently, filters, extracts or supernatants resulting from these assays had to be placed into individual LSC vials for analysis, resulting in high costs and low sample throughput.

We have developed the TopCount™ Microplate Scintillation and Luminescence Counter in response to the needs of investigators conducting many of these assay types. This instrument is a fully automated radioisotope and luminescence microplate counter, designed using the principle of all-reflective optics (Effertz, Neumann & Englert 1993; TopCount Topics 1992), which can assay up to 12 samples simultaneously. This design eliminates optical crosstalk effects, resulting in improved accuracy and dynamic range for both screening and quantitative assays.

We have also developed accessories specifically designed for counting samples isolated on filters. Experiments involving labels that may not remain firmly attached to the filter after addition of scintillation fluid must be counted on filter disks that are physically isolated from each other. To this end, samples can be collected using a Packard FilterMate™ harvester onto UniFilter™ plates, specially designed filtration plates containing discrete filter disks and integrated filter support screens. UniFilter™ plates can be counted directly on TopCount™. Alternatively, material collected using a commercially available filtration system manufactured by Brandel, Inc. (Gaithersburg, Maryland, USA) can be punched from a continuous filter sheet after harvesting into discrete microplate wells for counting. If large amounts of material are collected on a small surface area,

the filter can become clogged, and self-absorption effects can cause low and/or variable counting efficiency. To optimize reproducibility and maximize throughput, we have developed several methods of harvesting and counting on small or large discrete filters or continuous filter sheets, depending on the amount and type of material to be processed.

With a number of biological assays, it is also possible to use a microplate well, rather than a filter, as a solid support to immobilize cells or specific molecules. Examples include coated-well radioimmunoassay (RIA), adherent cell proliferation and cellular receptor binding. The microplate itself can be treated using a number of commercially available processes to promote cell adhesion and to specifically bind target molecules. After simple washing, the plate can be counted directly in the TopCount™, thus eliminating tedious punching and harvesting steps.

We have conducted receptor binding, cell proliferation and in-plate assays on TopCount™ with excellent results. We describe here new equipment that we used to prepare and count microplate samples, as well as the results of our experiments.

## METHODS

All reference assays were carried out on a Tri-Carb® 2500TR LSC or Cobra™  $\gamma$  counter (Packard). To evaluate performance for receptor binding assays, we used the  $^3\text{H}$ -benzodiazepine (Cat. No. NED-002) and  $^{125}\text{I}$ -endothelin-1 (Cat. No. NED-009) NENQUEST™ Drug Discovery Systems (DuPont-NEN Research Products, Boston, Massachusetts, USA). We carried out all sample preparation and incubation steps according to the kit instructions.

To test new accessories, we used a modular FilterMate™ cell harvester (Packard). With appropriate accessories, this harvester can be configured to process all samples simultaneously in either 24- or 96-well formats. It is designed to accommodate the UniFilter™ filtration plates that contain discrete glass fiber filter disks in individual wells, simulating a discrete filter in an LS vial. The diameters of the disks are 14 mm and 7 mm for 24- and 96-well plates, respectively. UniFilter™ plates are available with GF/B™ and GF/C™ (Whatman) filter materials.

As our reference filtration method, we used the Brandel 24-well (4 × 6) cell harvester (Model No. MPR-24) and filter punch/deposit device (Model No. MPDR-24), which deposits each of the 24 filter disks simultaneously into wells of a 24-well microplate. Filters so placed can be counted directly in the TopCount™ or removed for assay in a conventional LSC.

We processed standard competition curves in triplicate using the FilterMate™ cell harvester and both 24- and 96-well UniFilter™ filtration plates, each containing GF/B™ filter disks. After harvesting, we dried the plates and added MicroScint™ scintillation cocktail to each well (125 and 25  $\mu\text{l}$ , respectively). Finally, we counted each UniFilter™ plate on TopCount™ using the appropriate preset counting conditions. For a control assay, we processed an additional curve using the Brandel equipment. The 24 samples were transferred into a 24-well microplate using the Brandel punch system described above. We added 125  $\mu\text{l}$  of MicroScint™ cocktail to each well and counted the plate in the TopCount™. We then removed, solubilized and assayed the filters for absolute activity (dpm) in the discrete-vial LSC. Total count samples were assayed similarly.

We also investigated the feasibility of counting discrete-membrane microplates directly in the TopCount™. Here, we used white Multiscreen® HV filtration plates (Millipore Corporation, Bedford, Massachusetts, USA, Cat. No. SA2M060E2) and a Multiscreen® vacuum manifold. These devices are also designed for biological assays such as enzyme activity, cell growth and receptor binding. We set up duplicate competition curves for the  $^{125}\text{I}$ -endothelin kit on two plates. After

processing, one plate was attached to a reflective backing plate. We added 10  $\mu\text{l}$  MicroScint™ cocktail to each well and counted the plate in the TopCount™. The samples on the remaining control plate were punched into 12  $\times$  75-mm  $\gamma$  tubes using the Multiscreen® punch device and counted in the  $\gamma$  counter.

For cell proliferation assays, we used mouse spleen cells cultured to  $10^6$  cells/ml in a standard 96-well culture plate. After growth, the cells were stimulated with increasing amounts of anti-CD3. Prior to harvesting, the cells were pulsed with 266 Mbq (2 Uci)  $^3\text{H}$ -thymidine per well. We harvested all samples simultaneously into a UniFilter™-96 plate (GF/C™), using the FilterMate™ cell harvester, dried the plate, added MicroScint™ cocktail (25  $\mu\text{l}$ ) to each well and counted the plate on the TopCount™. Finally, we removed each of the filter disks and placed them into small LSC vials. We then solubilized the samples and assayed them for dpm on our conventional LSC.

To demonstrate performance for in-plate assays, such as coated-well RIA or cellular receptor binding, we chose the  $^{125}\text{I}$ -TSH IRMA assay. We first coated a solvent-resistant microplate (PicoPlate™, Packard, Cat. No. 6005162), a commercially available white microplate (Microlite™, Dynatech Laboratories, Chantilly, Virginia, USA, Cat. No. 011-010-7411) and white strip wells (Microlite™ Remov-a-well, Dynatech) with a universal solid phase consisting of ovalbumin/biotin and streptavidin. We then added diluted anti-TSH/biotin conjugate (Dr. Alain Baret, Laboratoire Trichereau, Nantes, France) to each well. After incubation and washing, we incubated TSH standards and  $^{125}\text{I}$  anti-TSH antibody (Amersham Corporation, Arlington Heights, Illinois, USA) with the first antibody. We washed the microplates again, and added 300  $\mu\text{l}$ /well of MicroScint™ cocktail to each well prior to counting on TopCount™. Finally, we washed the strips, broke them into discrete wells and counted each well in the  $\gamma$  counter.

## RESULTS AND DISCUSSION

### Receptor Binding Assays

Receptor binding assays conducted on tissue homogenates, whole cells or cloned receptors generally require the use of discrete filters, because radioligands bound to the filter tend to elute or migrate in the scintillation fluid, or require solubilization. We investigated several alternatives for counting on discrete filters. To evaluate the performance of TopCount™ compared to a traditional LSC using conventional methods, we compared the counting results for the samples processed with the Brandel equipment. We first counted the discrete filters on the TopCount™ and then placed them into LS vials. The radioligand was eluted off the filters using 1 ml of tissue solubilizer. After adding cocktail and mixing, each vial was assayed for dpm on our LSC. We calculated the TopCount™ counting efficiency by dividing the TopCount™ cpm values by the LSC dpm values. We found that TopCount™ gave an absolute  $^3\text{H}$  counting efficiency of 54%, with a correlation coefficient of 0.99. This indicates excellent response linearity, as well as high efficiency. We also calculated the percent bound, and plotted it against dose for each of the standard points (Fig. 1A).

The superimposed competition curves also demonstrate equivalent counting performance. We evaluated the data from each of the UniFilter™ experiments in a similar fashion. Figure 1B illustrates the competition curves for samples on the UniFilter™-96 plate and control samples processed with the Brandel equipment. Again, we observed excellent superposition. Finally, after converting all counting results to dpm, we calculated  $K_d$  and  $B_{\text{max}}$  for each experiment by conducting a Scatchard analysis. Table 1 gives a summary of all results. Relative efficiency is defined as the cpm obtained on TopCount™ divided by the cpm obtained for that sample counted on our LSC.

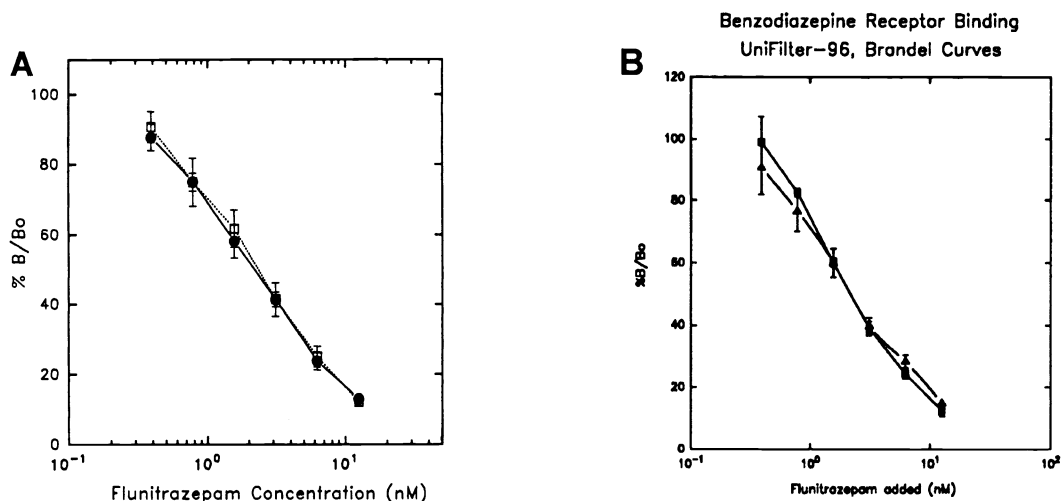


Fig. 1. Benzodiazepine receptor assay. A. Competition curves processed on Brandel equipment and counted on TopCount™ and conventional LSC. ● = TopCount™; □ = LSC. B. Competition curves processed on Brandel equipment (counted on conventional LSC and FilterMate® harvester/UniFilter®-96 filtration plates (counted on TopCount™). ▲ = TopCount™; ■ = LSC.

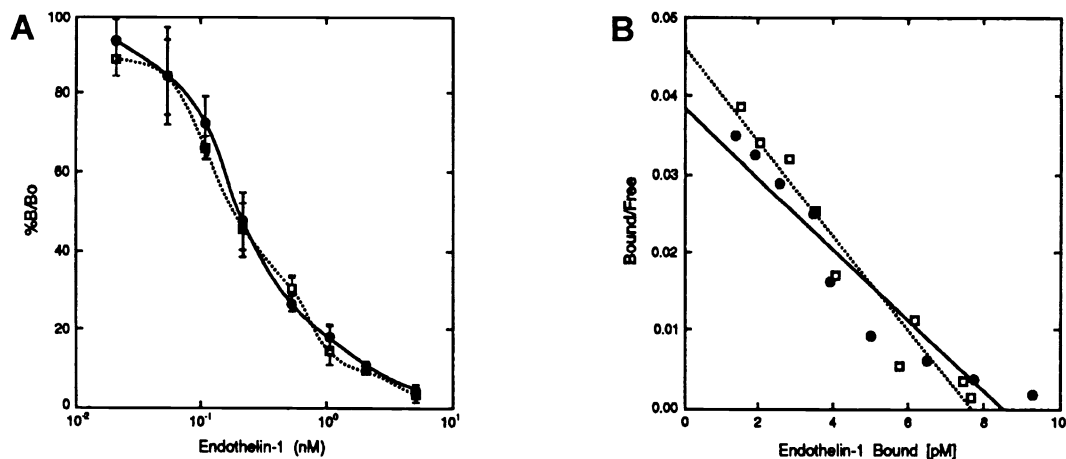


Fig. 2. Endothelin-1 receptor assay. A. Competition curves processed using Multiscreen® filtration plates and counted on TopCount™ and γ counter. B. Scatchard plots of the same data. ● = TopCount™; □ = γ counter.

Relative efficiency is based on cpm obtained on a traditional LSC. Literature values (DuPont-NEN data sheet) for the benzodiazepine receptor system are  $K_d = 1.4$  nM and  $B_{max} = 0.25$  nM. These results provide conclusive evidence that several combinations of harvesting equipment and filtration plates, in conjunction with the TopCount™ instrument, can be used to process and analyze accurately and rapidly receptor binding samples isolated on glass-fiber filter media.

We carried out a similar evaluation for the  $^{125}\text{I}$ -endothelin receptor kit using the Multiscreen® filtration plate system. Figure 2 illustrates the competition curves and Scatchard plots from the TopCount™ and γ counter assays. Again, we observed excellent agreement between the TopCount™ and control experiments.  $K_d$  and  $B_{max}$  values agree closely with published results (data

TABLE 1. Summary of Receptor Binding Experiments

Experiment	Relative Efficiency	Correlation ( $R^2$ )	Kd	Bmax
Brandel Punch/Deposit System	119%	0.99	1.3 nM	0.21 nM
FilterMate™/UniFilter™-24	90%	0.96	1.7 nM	0.20 nM
FilterMate™/UniFilter™-96	56%	0.98	1.8 nM	0.22 nM

not shown), which further demonstrates the feasibility of counting Multiscreen® plates directly in TopCount™.

### Cell Proliferation Assays

The ability to harvest samples rapidly onto discrete or continuous filters of 7- or 14-mm diameter is important. The UniFilter™ plates used for receptor binding assays also are ideally suited for cell proliferation. We processed the results for the cell proliferation experiments by correlating the UniFilter™/TopCount™ data (cpm) with that of the LSC. The correlation coefficient ( $R^2 = 0.99$ ) was excellent and absolute  $^3\text{H}$  counting efficiency was 28%, despite the fact that the radiolabeled material was not solubilized off the filter. We plotted the dose-response curves from both instruments (Fig. 3). The results are identical, within experimental error. They demonstrate that the FilterMate™/UniFilter™ harvesting equipment can be used with TopCount™ to produce results for cell proliferation studies quickly and efficiently, indistinguishable from those produced with conventional equipment.

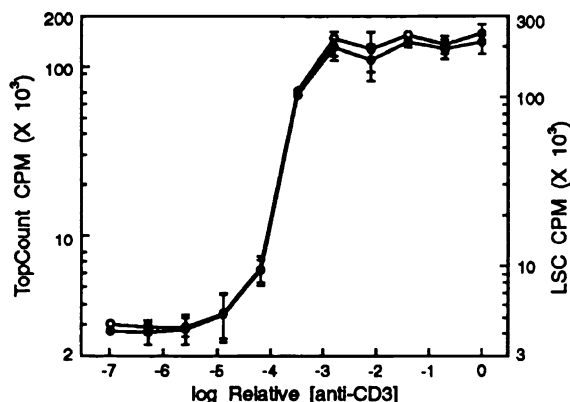


Fig. 3. Cell proliferation assay dose-response curves processed using FilterMate™ harvester/UniFilter™ filtration plates and counted on TopCount™ and conventional LSC

### In-Plate Assays

For in-plate assays, the ability to discriminate and quantitate samples accurately in the absence of crosstalk is critical. The use of all-reflective optics permits excellent quantitation with either isotopic or luminescent detection. We evaluated the feasibility of carrying out assays in which the radiolabel is specifically bound to the microplate well. We correlated TopCount™ counting performance using the TSH samples prepared in the Microlite plate, for TopCount™, and the Microlite strips for the  $\gamma$  counter (Fig. 4A), and found the correlation coefficient to be 0.99, with a relative counting efficiency of close to 100%. Figure 4B illustrates the dose-response curves for both types of microplates tested in the TopCount™. Essentially, the curves are identical, indicating

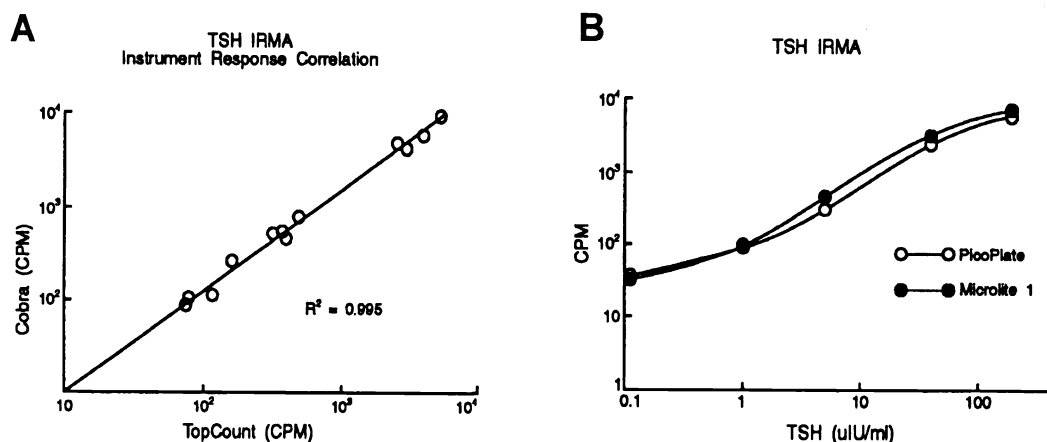


Fig. 4. TSH IRMA assay. A. Correlation of coated-well samples counted on TopCount™ and  $\gamma$  counter. B. Dose-response curves of Microlite™ and PicoPlate™ TSH samples counted on TopCount™.

that several different microplate supports can be used to produce excellent assay results. The use of microplate wells as solid supports is becoming increasingly popular for RIA/IRMA as well as receptor binding applications, where cell surface receptors are characterized by immobilizing cells on the microplate and specifically binding a radioligand to them. These results show that the TopCount™ can count these samples accurately with increased throughput and decreased labor.

## CONCLUSIONS

We have demonstrated that the TopCount™ Microplate Scintillation and Luminescence Counter is a suitable alternative to conventional LSC for processing a wide variety of samples produced during biological and pharmaceutical research. Accessory supplies and equipment, such as UniFilter™ plates and the FilterMate™ harvester, were designed specifically to accommodate the varying needs of these samples without compromising assay reliability or requiring unconventional processing schemes. We obtained excellent results for receptor binding, cell proliferation and *in-situ* microplate assays. Many of these samples are isolated on solid supports, such as filters, membranes or microplate wells. Depending on the specific assay, several alternative sample preparation methods can be used for TopCount™. Regardless of the method, the resulting samples can be accurately screened and quantitated directly in the TopCount™ instrument with improved sample handling, increased net throughput and decreased costs.

## REFERENCES

- Effertz, B., Neumann, K. and Englert, D. 1993 Single photomultiplier technology for scintillation counting in microplates. This volume.
- TopCount Topics #13 1992 *Crosstalk*. Meriden, Connecticut, Packard Instrument Company.