

# TopCount *Topics*

TCA-009

## Cell Proliferation Assays

### Abstract

The cell proliferation assay is an important tool in the assessment of the immune system function and in the search for new drugs. Productivity with this technique has been limited by the lack of automated, high throughput sample processing and counting equipment. The MicroMate harvester and the TopCount Microplate Scintillation Counter help remove these bottlenecks. Results from a cell proliferation assay are presented which compare the performance of TopCount to the traditional counting method.

### Introduction

Cell proliferation assays are employed frequently in immunological, cancer and pharmaceutical research to assess the ability of both natural and synthetic compounds to stimulate or inhibit proliferation of lymphocytes. Common growth factors or mitogens include lectins (*e.g.*, ConA, PWM), interleukins, and antibodies (*e.g.*, anti-CD3). Pharmaceutical agents may suppress the action of these compounds. Proliferation assays with mixed lymphocyte cultures or MLC's are also commonly used to evaluate the compatibility of donor and recipient tissues for organ transplants.

Cell proliferation assays measure the incorporation of a radiolabeled DNA precursor, [<sup>3</sup>H]-thymidine, into the replicating strands of DNA produced during cell division. Cultures are typically set up in 96-well microplates. The labeled DNA is usually captured with a cell harvester on glass fiber filter disks, which are then placed in liquid scintillation counting (LSC) vials for counting. These proce-

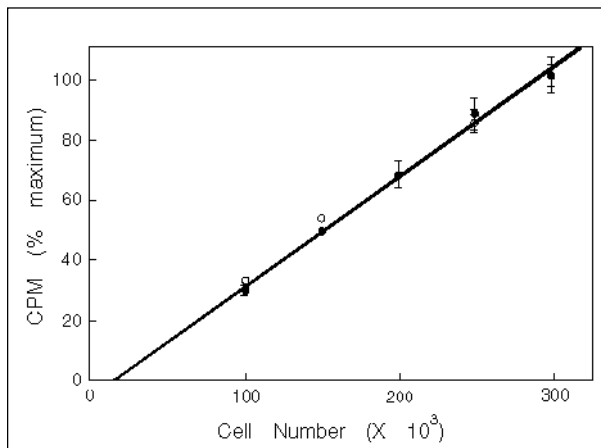
dures are both time and cost intensive. Transfer and processing of the samples in 96 discrete LSC vials limits assay throughput and drives up assay costs.

The MicroMate 196 Cell Harvester offers the ability to harvest 96 samples simultaneously into a standard 8 X 12 format filter plate assembly. Scintillation cocktail is then dispensed onto each filter using pipetting equipment designed for microplates. After sealing the plate, it can be loaded and counted directly in the TopCount Microplate Scintillation Counter. With up to 12 simultaneously counting detectors, TopCount can cut counting time to 10% of the time required by traditional liquid scintillation counting and decrease radioactive waste.

### Experimental

In an experiment to determine the response linearity of TopCount with increasing cell numbers, mouse spleen cells were cultured to a level of approximately 10<sup>6</sup> cells/ml and stimulated with anti-CD3. The cells were pulsed with approximately 2mCi of [<sup>3</sup>H]-thymidine for 24 hours, and then increasing volumes of the culture were dispensed into individual microplate wells so that the cell number per well ranged from 10<sup>5</sup> to 3 X 10<sup>5</sup>. Two plates were prepared, one to be counted on TopCount and another on a discrete vial LSC.

The cells were then harvested onto a Packard UniFilter plate using a MicroMate 196 Cell Harvester. The UniFilter plate is a special microplate, containing a glass fiber membrane and designed to be compatible with the MicroMate 196 Cell Harvester and TopCount. The filters are physically



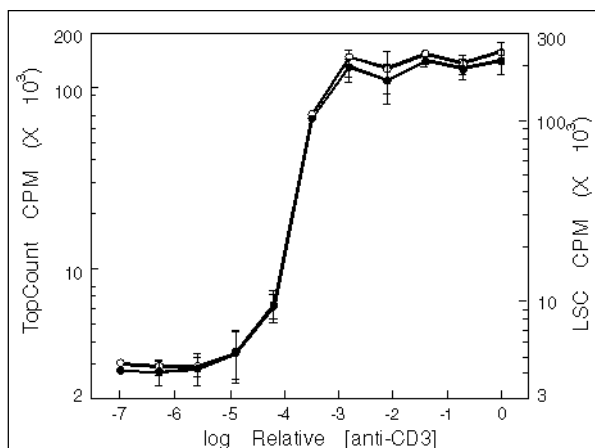
**Figure 1.**

Count rate for varying numbers of 3H-labeled mouse splenocytes measured with TopCount (•) and with conventional LSC (o). The linear regression line ( $R^2 = 0.995$ ) is fit to the TopCount data.

isolated from each other to prevent sample and cocktail migration and to eliminate optical crosstalk between wells. After drying, a reflective backing plate was attached to the bottom of one of the UniFilter plates, and 20 mL MicroScint-O was added to each well. The plate was sealed and counted on TopCount. The individual wells of the other UniFilter plate were removed, placed into 7 ml LSC vials, solubilized, and counted in a conventional LSC.

The count rates increased linearly with cell number (Figure 1). Thus TopCount provides accurate and consistent counting results for cell proliferation assays, across the normal range of cell numbers used in cell proliferation experiments.

A lymphocyte stimulation assay was performed in another experiment on TopCount. Mouse spleen cells were cultured to approximately  $10^6$  cells/ml in a 96-well cell culture plate and stimulated with increasing amounts of anti-CD3. As in the first experiment, all wells were harvested simultaneously into a UniFilter plate using the MicroMate 196 Cell Harvester. After processing and counting on the TopCount, all individual filter disks were removed from the filter plate, solubilized, and counted in a conventional LSC.



**Figure 2.**

Count rate measured with TopCount (•, left axis) and conventional LSC (o, right axis) for lymphocyte stimulation experiment.

The CPM results from the two instruments correlated well ( $R^2 = 0.994$ ). The TopCount efficiency was 28%, despite the fact that the DNA was not solubilized off the filter. The LSC efficiency with solubilization of the DNA into the scintillation fluid was 45%. The dose-response curves from the assay for both TopCount and LSC are shown in Figure 2. The results are identical within experimental error.

## Conclusions

Cell proliferation assays have been performed with the MicroMate 196 Cell Harvester and TopCount, using Packard UniFilter plates. The experiments show that results obtained on TopCount will be identical to those obtained on traditional liquid scintillation counters. Since the filter disks are optically and physically isolated, sample and cocktail migration, as well as optical crosstalk, are eliminated. The TopCount Microplate Scintillation Counter can reduce manual labor, increase throughput, and decrease costs of cell proliferation assays.