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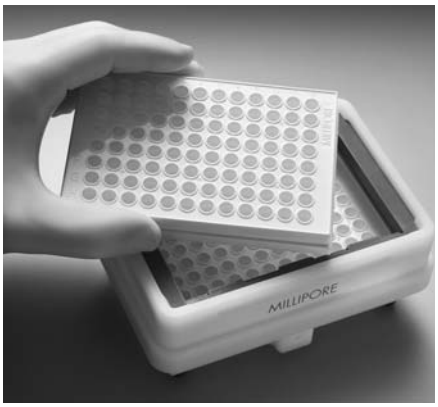
MultiScreen® Methods

Efficient Low-Energy Radioisotope Detection in Vial-based Liquid Scintillation Counting Using the MultiScreen Assay System



Filtration and Collection ■

The MultiScreen Assay System is a rapid, multiple-sample filter-based system for applications that range from ligand binding and cell-based assays to the screening of large numbers of low-volume samples. It can be used to perform many phases of a typical assay procedure, including sample preparation, incubations, washings, filter punching, and filter removal for radioisotope counting. It not only collects the precipitate for analysis, but can also be used to collect and quantify filtrate for use in determining bound versus free ligand, as in Scatchard analysis.[1,2,3]

Consisting of a 96-well filtration plate, a vacuum manifold for washing, and a membrane punch and collection unit, the versatile MultiScreen system is engineered for well-to-well consistency, filtration efficiency, and punching precision. Tight punching tolerances assure uniformity and maximum filter collection. Features that prevent cross-contamination and increase assay reliability include a removable underdrain that funnels filtrate into the wells and a punching system that uses disposable tips.

MultiScreen filter plates are available with a wide variety of membranes, including low binding Durapore®, glass fiber filters and mixed cellulose esters. The Durapore PVDF membrane is available in four pore sizes: 0.22 µm (GV), 0.45 µm (HV), 0.65 µm DV, and 1.2 µm (BV). The membrane is non-toxic to living cells[3] and exhibits extremely low-non-specific protein binding[4] (NSB), broad chemical compatibility, and reliability for critical biological separations.

Other filter materials such as high protein-binding nitrocellulose (0.45 µm, HA) and Immobilon™ PVDF (IP), glass fiber filters (FB and FC), and phosphocellulose expand platform cababilities to include protein-binding, cell-based and kinase assays. Because the membranes are a durable, consistent matrix that is easily and cleanly punched, they repeatedly produce low coefficients of variation (CVs) in assay results. Each punch tip is exactly positioned for maximum precipitate collection.

In the MultiScreen system, the membrane with precipitate is punched and deposited—along with the disposable punch tip—directly into the tube or scintillation vial for counting. In most assays, radioisotopes such as ^{125}I that become trapped on the membrane are easily counted. In tritium-labelled assays, however, radiation from weak beta-emitting radioisotopes can become quenched by the filter, punch tip, or collected precipitate, affecting liquid scintillation counting (LSC) results (see Figure 1).

Proper assay procedures, however, can help eliminate this "beta absorption" quenching. This paper describes the results of experiments investigating factors that affect liquid scintillation counting with the MultiScreen system. It also presents an optimal LSC procedure for use with the system for maximizing tritium counting efficiency.

Detecting Beta Particles ■

Beta (ß-) emitting radioisotopes such as tritium are popular as labels in a variety of applications, such as thymidine uptake, cell-based assays, receptor binding assays, and immunoassays. However, their low level of energy can cause difficulties in liquid scintillation counting when samples are collected on any solid support, including a microporous membrane.

The difficulty arises because beta radiation, unlike gamma radiation, can travel only short distances before its energy dissipates. This presents no problem when tritiated samples are homogeneously dissolved in the scintillation cocktail. But with samples that have been precipitated onto a solid matrix such as a filter, the particles may be prevented from dispersing into the cocktail when it is added. They may be quenched by the filter or sample before they can even make contact with the cocktail solution. Consequently, the absorbed particles go undetected, leading to unreliable test results and incorrect conclusions.[5,6]

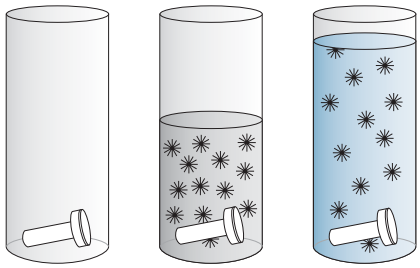
Reproducibility with ^{125}I-Labelled IgG ■

In order to establish punching precision, we studied the effect of three Durapore membrane types on TCA precipitate collection of IgG labelled with ^{125}I gamma radiation to eliminate any quenching variability that may occur with weak ß-emitting isotopes.

The nearly identical results in Table 1 show the high reproducibility of all three MultiScreen membranes in collecting the precipitates. The results also clearly illustrate how the precise punch system assures accurate and uniform filter collection. The CVs after punching are no greater than those observed after counting and pipetting operations only.

Figure 1. Releasing Tritium Emissions for Counting

1. Punch tip and filter deposited in vial (dry). *Tritium emissions may be absorbed into filter or sample before they can reach the scintillation cocktail.*
2. 30 minutes after adding water. *Thorough mixing with aqueous solution releases radiation for subsequent detection by the LS counter.*
3. Resolubilized precipitate in LSC and released tritium emissions.



Short Procedure

1. Collect and wash precipitate on MultiScreen plates.
2. Remove underdrain, blot excess liquid, and completely dry using heat lamp.
3. Punch out filters into vials.
4. Add 500 µL water and mix for 30 minutes.
5. Add LSC, shake, and count.

Table 1. Collection of TCA Precipitates — ^{125}I IgG

Membrane	Mean cpm (n=8)	±	S.D.	% CV
GV (0.22 µm)	13,738	±	376	2.74
HV (0.45 µm)	13,598	±	191	1.41
DV (0.65 µm)	13,575	±	240	1.77

Table 2. Liquid Scintillation Cocktails*

Name	Abbreviation	Manufacturer	Biodegradable	Flash Point °C
Ready Safe	RS	Beckman	Yes	163
Ready Protein	RP	Beckman	No	43
Filter Count	FC	Packard	No	50
Ultima Gold	UG	Packard	Yes	152
CytoScint	CS	ICN	Yes	155

*Information obtained from respective manufacturers' product literature.

Analyzing Variables Affecting Tritium Counting Efficiency ■

In these studies, we investigated five factors that influence tritium counting efficiency:

1. protein concentration
2. a) cocktail volume, b) water resolubilization of precipitate, and c) base resolubilization of precipitate
3. filter/punch sample mixing techniques
4. varieties of LSC solutions
5. filter plate membrane type

We used five commercial cocktails (Table 2) for generating data. However, the MultiScreen system is not limited to these brands and can also be used with many other available cocktails. All liquid scintillation counting was done on a Beckman Model LS5000TD counter.

A review of the five factors follows:
1) Protein concentration. Figure 2 shows that 1 mg/mL of bovine serum albumin (BSA) is the minimum protein concentration to produce a precipitate that can be effectively collected by the HV (0.45 µm) plate. Greater protein concentrations produce larger precipitates that can slow down the filtrate (supernatant) flow and at high concentrations even clog the membrane. Therefore, this minimum concentration was used in all experiments for greatest speed and highest collection efficiency.

Total counts represent the ^{3}H-DNA in the given BSA concentration in 500 µL water with 5 mL of Ready Safe cocktail added. The sample counts (n=8) represent the punched HV filter with collected trichloroacetic acid (TCA) precipitate added to 500 µL water, mixed for 30 minutes, and followed by the addition of 5 mL cocktail. The CVs for the 1 mg/mL concentration were 4.3%.

2) a) Cocktail volume. Many LSC users are not only converting to biodegradable, environmentally-safe cocktails, but also cutting back on cocktail volumes. Table 3A summarizes the results of experiments using our standard procedure with biodegradable Ready Safe cocktail. Varying the cocktail volume from 5 to 1 mL reduced the counting efficiency, although the reproducibility was not affected. (Supporting data is not shown.) Lower volumes of LSC can be used if lower counting efficiencies are tolerated by the assay system.

b) Water resolubilization of precipitate. Table 3B shows the results obtained when we varied the volume of water used to resolubilize the precipitates. We obtained the most consistent and optimal cpm recovery when we collected the filter/punch in the 500 µL of water before adding the 5 mL of cocktail. The table also illustrates that although a minimal amount of aqueous solution is required to dissolve the precipitate, lower volumes can be used if slightly higher CVs are within an acceptable range.

c) Base resolubilization of precipitate. Table 3C shows that 0.01 N potassium hydroxide is more effective at this low concentration than water in resolubilizing the tritium-labeled DNA from the membrane/punch. The potassium hydroxide may therefore be preferred for assays producing low counts due to factors such as low specific activity, low receptor density, or scarcity of label. However, use of elevated (0.4 N) potassium or sodium hydroxide levels can completely quench tritium emissions, resulting in no detectable cpm. (Supporting data is not shown.)

3) Filter/punch sample mixing techniques. Table 4 shows the results of experiments to determine the proper sample preparation method for the filter/punch before adding cocktail. Lowest cpm was generated when samples had no water added and were simply inverted by hand several times. Samples with 500 µL of water added and similar mixing produced triple the cpm. Adding the 500 µL

WARNING: *Never use elevated base (e.g., 0.4 N sodium or potassium hydroxide) concentrations with the MultiScreen system, regardless of assay protocol.*

Figure 2. Variations in Protein Concentration

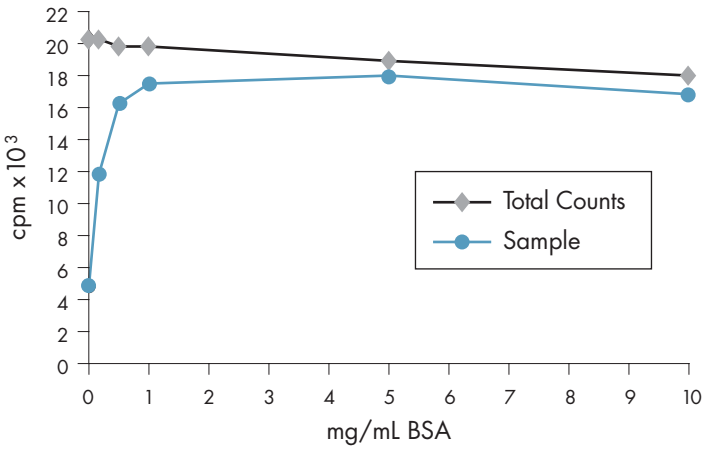


Table 3. Variables Affecting Counting Efficiency

A. **Ready Safe Cocktail Volume — Typical Performance**
5 mL of cocktail added to 0.5 mL of water
(24 wells analyzed in two different HV plates)

	cpm	±	S.D.	% CV
	16,049	±	856.4	5.3

B. **Punch/Filter Water Collection Volumes**
Constant Ready Safe cocktail volume of 5 mL
(16 wells analyzed in two different HV plates)

Water Volume	cpm	±	S.D.	% CV
100 µL	13,523	±	1,913	14.1
200 µL	15,542	±	2,206	14.2
300 µL	16,251	±	1,280	7.9
400 µL	16,461	±	914	5.5
500 µL	15,891	±	800	5.0

C. **Water vs. Base Resolubilization**
(8 wells tested with 5 mL of Ready Safe cocktail and 0.5 mL of solution on an HV plate)

Solution	cpm	±	S.D.	% CV
Water	15,621	±	1,036	6.6
0.01 N KOH	16,788	±	515	3.0

water and agitating in an orbital shaker for 30 minutes significantly increased cpm and subsequent counting efficiency. It is this combination of added water and continuous shaking that is the recommended sample preparation procedure.

Also shown in Table 4 are the NSBs (the non-specifically adsorbed cpm) that were detected when TCA was omitted in the precipitation for each method. These were prepared by replacing the 20% TCA addition with buffer.

4) Varieties of LSC solutions. The five LSC solutions in Table 2 were evaluated for general counting efficiency in detecting tritium radiation with the MultiScreen system.

The RP and FC cocktails are designed to solubilize precipitates and nitrocellulose or glass fiber filters commonly used in these procedures. They require, however, separate and expensive waste collection.

Table 5 shows that the cocktails designed for filter counting offer no significant advantages for counting the PVDF Durapore membranes. Overall, the ICN CytoScint LSC yielded the highest efficiencies for both total counts and samples of the filter/punch. The MultiScreen system, therefore, performs well using biodegradable cocktails with high tritium counting efficiencies.

5) Filter plate membrane types. All three Durapore membrane plates demonstrated low CVs when used with the recommended tritium counting procedure below. Figure 3 shows that the plate types performed similarly with each of the cocktails.

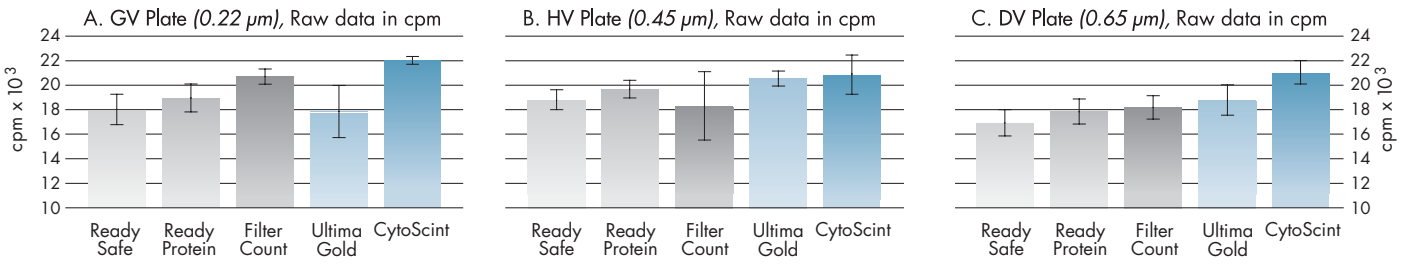
Table 4. Sample Preparation Methods
(8 wells evaluated using Ready Safe cocktail on an HV plate)

Method	cpm	±	S.D.	% CV
Dry/hand invert	4,049	±	287	7.1
NSB	193			
Wet/hand invert	12,895	±	1,200	9.3
NSB	165			
Wet/orbital shaker	18,174	±	574	3.1
NSB	150			

Table 5. LSC Efficiency and Effectiveness

LSC	Total cpm	Sample cpm (n=10)	±	S.D.	% CV
RS	16,616	16,946	±	423	2.4
RP	21,142	17,215	±	302	1.7
FC	20,401	17,168	±	230	1.3
UG	19,814	17,747	±	262	1.4
CS	27,315	21,041	±	261	1.2

Figure 3. LSC Comparisons, by Plate Type



High-Efficiency Procedure for Tritium Counting ■

The optimized procedure for collecting tritium-labelled protein or nucleic acids precipitated by trichloroacetic acid (TCA) on MultiScreen filter plates for LS counting follows. We ran this procedure using deoxyribonucleic acid (*E. coli*) (Thymidine-Methyl-^{3}H) supplied by Dupont's NEN Research products. The lot number was 2609-093, catalogue number NET-561, with specific activity of 11.2 µCi/µg and a concentration of 25 µCi/mL.

1. Add 100 µL of 1 mg/mL bovine serum albumin (BSA) solution in phosphate buffered saline (PBS, pH 7.4) containing 0.05 µCi activity/100 µL ^{3}H-DNA per well.

2. Add 100 µL ice cold 20% TCA per sample well (for non-specific binding controls, substitute 100 µL PBS buffer).

3. Incubate 30 minutes at 4 °C.

4. Vacuum and add 100 µL 10% TCA to wash the wells. Vacuum again.

5. Remove the underdrain from the MultiScreen plate and blot on paper towel or other suitable adsorbent material. Dry using a heat lamp. NOTE: For MultiScreen-PH, MultiScreen-HA and MultiScreen-DE, the filters should not be completely dried.

6. Load collection racks with appropriate scintillation vials. [Mount the 96-well disposable punch tip array on top of the filter plate with the points facing the membranes.]

7. Set the filter plate into the carrier slide and mount the 96-well disposable punch tip array on top of the filter plate with the points facing the membrane. NOTE: If performing the assay with MultiScreen$_{HTS}$ plates, the standard slide need to be replaced with Millipore part no. MSCP 096 00.

8. Insert the filter plate in the punching unit, then push it to the rear of the machine. Gently slide the rack forward until the first row of eight wells clicks into place. Firmly and smoothly pull the punch lever arm, depositing filters and punch tips into collection containers. Repeat this step until all filters have been punched and deposited.

9. Add 500 µL of water to the vial with the collected filter/punch tip and place on an orbital shaker for 30 minutes.

10. Add 5 mL of liquid scintillation cocktail to each tube or vial, replace covers, and invert several times to insure complete sample mixing.

11. Count samples with appropriate controls and determine results. Prepare total counts by adding 100 µL of working hot stock to 400 µL water, and then adding 5 mL of cocktail.

Summary ■

We have presented data and a sample procedure to help in designing your own procedures using the MultiScreen system for assays with tritium-labeled compounds. This procedure could also be applied to liquid scintillation counting with higher energy radio-isotopes such as ^{14}C, ^{32}P, and ^{35}S.

This procedure is based on findings that illustrate the accuracy and precision of the MultiScreen system with the proper volumes of cocktail and water for dissolving the precipitate, and with thorough mixing. An optimum concentration of protein (1 mg/mL) assures highest counting efficiency at the greatest flow rate through the membrane. The precise determination of tritium-counting efficiency with solid-phase samples is extremely difficult. The tritium-counting efficiency obtained using the MultiScreen system and the procedure recommended here was consistently between 28%–40%, depending upon the choice of liquid scintillation cocktail.

Results also showed that with this procedure, the MultiScreen system produces extremely low CVs, with a level of variability so small it may be attributed to pipetting or counting error. Recommended for use in all types of binding assays, it is economical and reliable for screening drugs, cells, or other unknown components because it rapidly processes a large number of small samples. The membrane in the filtration plate is biocompatible, exhibits low protein/nucleic acid binding, and works well with all scintillation cocktails tested. The precise punching system regulates the collection of filters and the underdrain regulates the collection of filtrates for a higher degree of confidence.

The MultiScreen assay system is a reliable, reproducible, and convenient means of separation and sample handling for a wide range of applications.

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