Filter counting by LSC.

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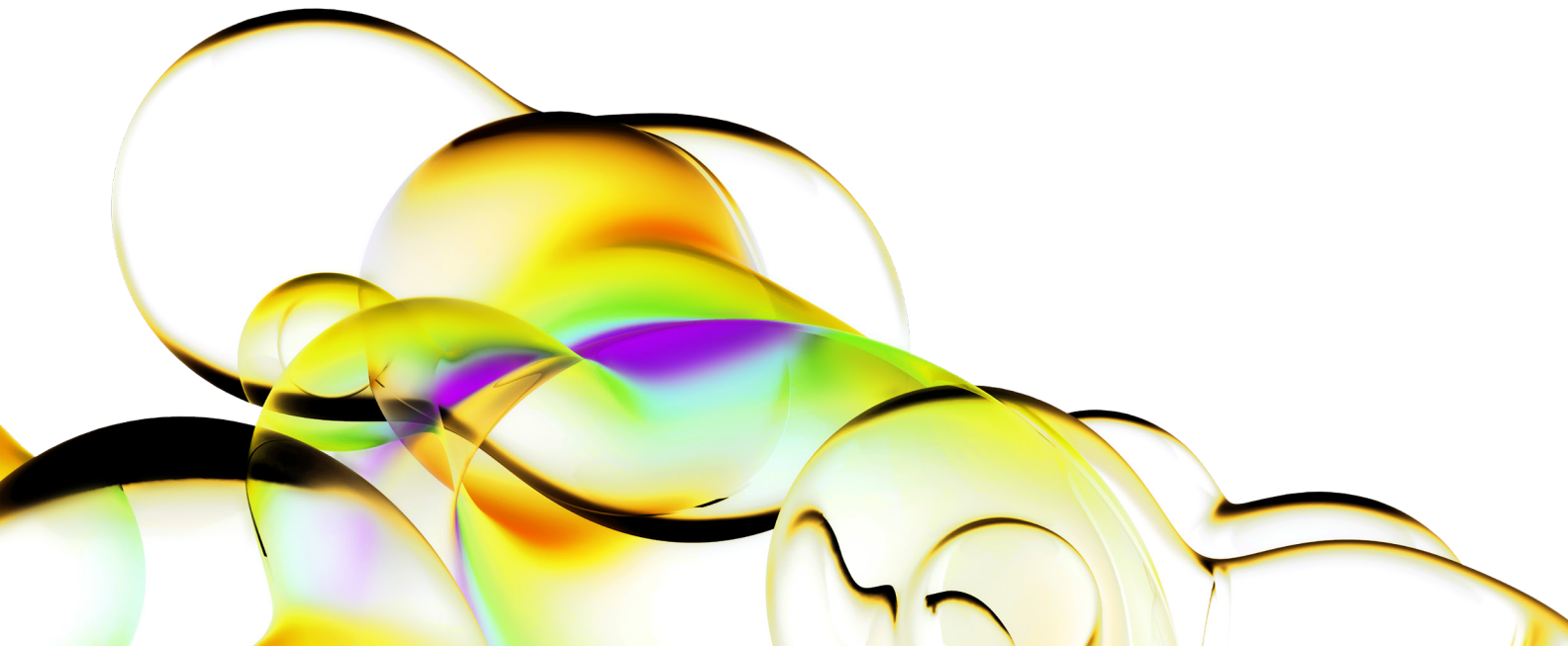
Introduction

Filter counting, or solid support counting as it is sometimes known, is probably best described as heterogeneous counting. The main difference between heterogeneous counting and homogeneous counting is that heterogeneous counting relies on 2 p geometry while 4 p geometry applies to homogeneous counting. An explanation of the terms 2 p and 4 p geometry is needed to appreciate the differences between the two counting techniques. In homogeneous counting, the sample is completely "dissolved" in the liquid scintillation cocktail; therefore the photons of light (scintillations), which are emitted as the end result of the energy transfer process within the liquid scintillation cocktail, are free to radiate in any direction. In geometric terms this freedom of radiation is described by a sphere or globe whose surface area is 4 pr^2. In heterogeneous counting, this freedom is restricted by the presence of the filter or membrane which absorbs both the kinetic energy of β-particles and the photons of light passing in one plane; therefore the emitted light can only occupy the surface area of a hemisphere which is 2 pr^2. Hence the derivation of the expressions 2 p and 4 p counting geometries and these are illustrated in Figure 1.



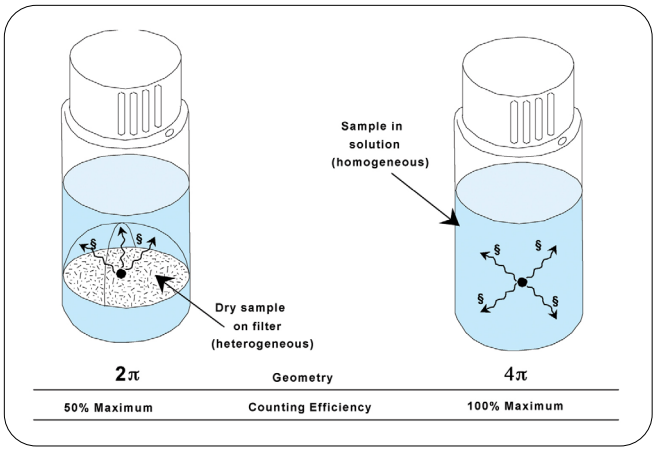


Figure 1: Sample counting geometries encountered in liquid scintillation analysis.

In essence filter counting can be a relatively simple technique where the sample is isolated or collected on a filter and usually dried. This filter is placed in a scintillation vial; a small volume of an appropriate LS cocktail such as Ultima Gold™ F is added and, after ensuring that the filter is completely wet, the vial is counted. The difficulty in counting on filters or other solid supports is that when the sample is immersed in a cocktail, four situations may develop:

1. The sample may remain bound to the filter or solid support – **no elution** situation.
2. The sample may be partially eluted by the cocktail – **partial elution** situation.
3. The sample may have a certain solubility in the cocktail – **equilibrium** situation.
4. The sample may be completely dissolved in the cocktail – **complete elution** situation.

Of these, the one to avoid is partial elution as the soluble fraction is counted with 4 p geometry, whereas the insoluble (filter bound) portion is counted with 2 p geometry. This will make measurement under these circumstances irreproducible. However, a partial elution situation may go to equilibrium with time and therefore, should not be discounted out of hand. Repeat counting of the sample, over several hours, will determine if an equilibrium situation has been reached and this is characterized by a constant count rate being obtained over time. If the sample is insoluble (no elution), the efficiency and reproducibility of counting will depend on the magnitude of the β-energy, the nature of the filter or solid support, its orientation in the vial, and the size of the sample molecule. If the sample is completely dissolved or eluted into the cocktail (complete elution), counting considerations will be similar to those of solubilized samples, where a true homogeneous state is obtained. Other factors which affect counting are the presence and composition of the sample precipitate and the amount of sample that becomes soluble in the cocktail[1].

Self-absorption affects the efficiency of counting on solid supports with low energy ^{3}H samples being more susceptible than higher energy isotope samples such as ^{14}C. The type and amount of sample and the thickness, absorption level and the material of construction of the solid support also influence the self-absorption effect. The order of counting efficiency of solid supports is: glass fiber > cellulose acetate > standard cellulose > chromatographic paper[2,3]. This order of efficiency will vary depending upon the size of the molecules. Smaller molecules can readily diffuse into amorphous regions of the cellulose fibers while the larger molecules may remain on the surface. Microscopically, glass fiber filters appear as an impermeable virtual network of threads whereas the paper filters appear as capillary tubes. For glass fiber filters, the efficiency can be markedly different for sample material trapped on the surface as opposed to that embedded in the pores. This is particularly true for low energy beta-particles from ^{3}H. In some cases, reproducible counting efficiencies can be obtained by addition of a carrier of known weight (many times more than the sample), which subsequently induces the same amount of self- absorption for each sample[4]. The carrier must be added before the filtration step, and time must be allowed for complete mixing with the real sample. When using chromatographic paper to isolate or collect samples, one should remember that some grades contain a UV enhancer and this can be eluted into the cocktail producing unwanted chemiluminescence. Such spurious counts can lead to an overestimation of the activity of the sample. Providing all of these factors and effects are taken into consideration during sample preparation, successful and reproducible counting can be accomplished using this technique.

In practice there are a number of filter types which can be used to isolate or collect various sample types for LSC analysis. The choice of filter type will depend upon both the nature and particle size of the sample, however glass-fiber filters are recommended if at all practical. Other filter types which have been used include:

- Cellulose nitrate
- Cellulose acetate
- Mixed cellulose esters
- Polyvinyl Chloride (PVC)

- Polyacrylonitrile
- Normal paper
- Polycarbonate
- PTFE
- Nylon
- PET

The categories of sample types which can be analyzed by this technique include:

- Precipitates of macromolecules (such as nucleic acids and proteins).
- Aquatic and terrestrial ecosystem samples (such as algae and phytoplankton).
- Other deposits (such as airborne particulate matter).

Sample preparation methods

The different elution situations influence both the choice of the sample preparation method and the recommended liquid scintillation cocktail.

1. No elution

This situation is highly desirable since sample preparation for counting by LSC is both simple and rapid. Sample quench is constant and simple CPM (counts per minute) mode on the LSC is preferred as external standard quench correction cannot be employed. With constant quench and therefore, constant efficiency, the CPM results obtained are as accurate as DPM (disintegrations per minute) results obtained through normal 4 p homogeneous counting. After collection of the sample on the filter, the filter is dried and placed in the vial. Approximately 2 - 3 mL of cocktail is added (ensuring that the filter is completely wet) and counting can be carried out immediately. For best counting performance using this method, it is recommended that the filter is completely dried prior to the addition of cocktail. Additionally, a knowledge of the solubility characteristics of the sample will aid in the selection of the most appropriate cocktail. In general, the most applicable cocktail for dried filter counting is Ultima Gold F, which provides the highest counting efficiency. Occasionally, it is not practical to completely dry the filters; and in these cases Ultima Gold MV should be used with the slightly damp filters for highest counting performance. The type of samples routinely counted using this method include precipitates from DNA and RNA studies, phytoplankton from sea water, algae from aquatic environments, as well as samples from enzyme activity assays, cell proliferation and receptor binding assays.

Notes

a. A simple method to confirm that a no elution situation exists is to decant the cocktail into another vial and recount the cocktail – absence of activity confirms that no elution has occurred and that the sample is completely bound to the filter.

b. If necessary, accurate quantitation of the total isotope activity (i.e., DPM) can be carried out by removing the filter and using either solubilization or combustion techniques.

2. Partial elution

As previously mentioned this situation is the least desirable due to the presence of both 2p and 4p geometry within the counting mixture. Any results from this situation will be inaccurate and cannot be reproduced. It is possible however, using one or a combination of the following methods, to convert the system from partial to an equilibrium or complete elution situation:

a. After sample preparation shake the contents for a fixed time period and recount. Repeat this procedure until constant CPM's are obtained, i.e., equilibrium situation.

b. Change to a cocktail in which the sample has either zero or complete solubility.

c. Extract the sample from the filter with a suitable solvent prior to adding the appropriate cocktail.

3. Complete elution

The goal with complete elution is to convert from 2 p to 4 p geometry. Two slightly different sample preparation methods are employed in that either the entire filter is dissolved in an appropriate cocktail or the sample is extracted/eluted from the filter prior to the addition of cocktail. In the first instance the cocktail of choice is Filter-Count™ and the following filter types can be dissolved by this cocktail:

- Cellulose nitrate
- Mixed cellulose esters
- Polyvinyl Chloride(PVC)

Filter-Count will not dissolve cellulose acetate, glass fiber, normal paper, PTFE, nylon or phosphocellulose filters. With cellulose acetate and glass fiber filters a transparent appearance results, while the others remain relatively unaltered. Filter- Count will not give color formation with any filter whether soluble or insoluble.

The use of Filter-Count is extremely simple in that sample preparation involves adding cocktail (Filter-Count) to the filter, allowing it to dissolve (with optional heating) and then counting. When using cellulose acetate, the recommended reagent is Soluene®-350 which completely dissolves this filter type. After dissolution, the LSC cocktail Hionic-Fluor™ is recommended for trouble free counting. It is important to note that with the exception of cellulose acetate, normal paper and PET filters, virtually all other filters produce color when used with Soluene-350. In this variant of the technique, dissolving, dissolving the filter overcomes the self-absorption problems, saves on drying time (accepts wet or dry filters) and provides reproducible results. In the second case, as previously described, the sample is extracted or eluted from the filter with a suitable solvent and then counted using the appropriate cocktail.

Note

It is possible to adapt this technique for alpha/beta counting of airborne particulates. Providing the correct filter type is used the sample filter can be dissolved in Filter-Count; then Ultima Gold AB can be added (ratio of 2:1 Filter-Count:Ultima Gold AB). The benefits of such a method include the removal of self-absorption problems (especially important for alphas) and significant time saved on sample preparation (ashing and acid extraction steps are eliminated).

Recent developments

The recent introduction of the TopCount® Microplate Scintillation and Luminescence Counter together with the development of various filter plates offers the ability to count labeled samples in filter plates (24 or 96 samples per plate), minimizing sample preparation steps and increasing sample throughput. A number of publications on the applicability of TopCount for this assay method are available[5,6,7].

Summary

The information presented in the previous sections of this publication are condensed into a quick reference table (Table 1). This table can be used as a guide for selecting the correct LSC cocktail for a particular filter or membrane type.

Conclusion

There are a variety of Revvity LSC cocktails which are suitable for filter and membrane counting. If problems with counting on solid supports persist, please call your local Revvity representative for further applications support.

References

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4. Wang, C. H. and Willis, D. L., *Radiotracer Methodology in Biological Science,* Prentice-Hall, Inc., New Jersey, p. 202-206, 1965.
5. TopCount Topics #11, Direct Counting of Millipore® MultiScreen® Filtration Plates, Revvity, Inc.
6. TopCount Topics #12, Biological Applications of Microplate Scintillation Counting, Revvity, Inc.
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Filter type		Filter-Count	Ultima Gold F	Ultima Gold MV	Soluene-350 + Hionic-Fluor	Filter-Count + Ultima Gold AB
Glass Fiber	Dry	✓	✓	✓		✓
	Wet	✓		✓		✓
	Dissolved					
Cellulose Nitrate	Dry		✓	✓		
	Wet			✓		
	Dissolved	✓				✓
Cellulose Acetate	Dry	✓	✓	✓		✓
	Wet	✓		✓		✓
	Dissolved				✓	
Mixed Cellulose Esters	Dry		✓	✓		
	Wet			✓		
	Dissolved	✓				✓
PVC	Dry		✓	✓		
	Wet			✓		
	Dissolved	✓				✓
Polyacrylonitrile	Dry	✓	✓	✓		✓
	Wet	✓		✓		✓
	Dissolved					
Polycarbonate	Dry	✓	✓	✓		✓
	Wet	✓		✓		✓
	Dissolved					
PTFE	Dry	✓	✓	✓		✓
	Wet	✓		✓		✓
	Dissolved					
Nylon	Dry	✓	✓	✓		✓
	Wet	✓		✓		✓
	Dissolved					
PET	Dry	✓	✓	✓	✓	✓
	Wet	✓		✓	✓	✓
	Dissolved					
Normal Paper	Dry	✓	✓	✓	✓	✓
	Wet	✓		✓	✓	✓
	Dissolved					