May 28, 1996

Ligand Binding Experiments With Aqueous Cell Suspensions

Problem:
A researcher was performing ligand binding experiments and asked for assistance to select a new, safer cocktail for processing his 30,000 samples per year. The researcher wished to avoid the time consuming task of testing a wide variety of cocktails and turned to Packard for a solution.

The researcher's ligand binding assays were performed on aqueous cell suspensions. The procedure involved harvesting of the samples onto glass fiber filters without drying. The total filtered tissue samples contained approximately 200 - 400 µg of protein.

Discussion:
There are three general approaches that may be considered for this type of assay and the number of samples that are being processed annually. The three approaches are: solubilization, elution and conversion to a microplate-based methodology. These techniques are discussed below.

Solubilization: Packard's Solvable™ (Packard part number 6NE9100) is an aqueous-based solubilizer designed for digestion of materials such as tissue samples and protein. The basic procedure would involve solubilization of the sample into a homogeneous solution, decanting of the supernatant and counting using one of Packard's safer scintillation cocktails. The primary concern when conducting solubilization is the possibility of chemiluminescence due to the addition of the highly basic solubilizer to the liquid scintillation cocktail.

We tested and confirmed that 10 mL of the following cocktails can be used with up to 1 mL of Solvable with little, or no, chemiluminescence produced.

Cocktail	Packard Part Number
Ultima Gold™	6013329
Ultima Gold XR	6013119
Ultima Gold MV	6013159
Opti-Fluor®	6013199

Elution: Depending upon the exact genus of the cell sample, it may be possible to use Ultima Gold MV to perform elution. We know of at least one researcher that adds the damp filter to a vial and then adds cocktail, mixes and allows it to stand for predetermined periods of time. The mixing and standing steps ensure that all samples reach a stable, or constant, elution situation.

Method Conversion: With the high number of samples processed by this researcher, it could be very cost effective and efficient to convert to a microplate-based assay on a Packard TopCount™ microplate scintillation and luminescence counter. The researcher could process and count 24 or 96 samples at a time using a Packard Filtermate™ harvester and a TopCount with UniFilter™ microplates.

Recommendation:
The first approach, solubilization, may be the fastest method for the researcher to adopt. However, we recommend conversion to microplate harvesting and counting with a Packard TopCount as the most practical, long-term solution.

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