8 Miscellaneous Tips

Miscellaneous Tips Worth Repeating

- Always use the same fluorimeter with the same machine settings and the same set of cuvettes for all samples from a single experiment.

- The fluorescence intensity of a sample can be increased by three means:

  1) injecting more microspheres,
  2) increasing the excitation and/or emission slit widths, or
  3) using less solvent will increase the fluorescent dye concentration.

  Each of these approaches has advantages and disadvantages.

- If the solution to be measured is visibly colored, the fluorescence from that sample will most likely be too high.

- Meticulous cleanliness is mandatory when using optical instrumentation. Use talc-free gloves whenever handling cuvettes. Cuvettes should be washed on a regular basis. Cleaning methods suggested by a cuvette manufacturer (Starna, CA) are as follows:

  Cuvette Cleaning Methods

  Most laboratory detergents may be used at recommended concentrations; however, if the pH is greater than 8.5, etching may occur with repeated use. In general, neutral detergents such as 'Neutracon' are safe. We use ES™ 7X® Cleaning Solution from ICN Biomedicals, Inc., diluted 1:100 in distilled water.

  Rinse with distilled water followed by analytical grade methanol and/or acetone.

  These cleaning methods should never be used on cells that are not of fully-fused construction, as most of these reagents will attack the adhesives at the interface between the optical surfaces and the cell body.

- Tween-80® can cause transient hypotension in certain animals, depending on the concentration of Tween-80® and the rate of injection. If this is a problem, the microspheres can be centrifuged, the supernatant poured off, and the microspheres resuspended in saline. A small amount of Tween-80® should be used to prevent aggregation of the spheres.

- Suitable membrane filters are available from several manufacturers, including Millipore Corp., Bedford, MA and Poretics, Inc., Livermore, CA. In some cases, the filters have precisely sized holes that are made by charged particles from a nuclear reactor. Filters are available in several sizes and the filtration apparatus, which includes a glass support, a clamping device and a vacuum funnel, can be purchased from the filter suppliers. The filters should be stable to 4N KOH for a brief period of time (see Section 5).

- Do not use other tissue dyes in samples, as they may absorb the excitation and or emission light when measuring fluorescence (see Section 2 regarding vital dyes).

- If fluorescent dye solutions are too concentrated, quenching (reduction) of the fluorescent signal can occur, leading to incorrect results. Usually, solutions will be dilute enough to prevent this, but suspected quenching can be confirmed by diluting the sample 1:1 in the extraction solvent. The fluorescence reading should be 50% of the original solution if quenching is not present. If quenching is present, the diluted solution will have greater than 50% of the original solution (see Section 3).

- At equal concentrations, some fluorescent microspheres emit a greater signal intensity than others when the recommended wavelength pairs from Table 1 are used (see Figure 1). It is possible to decrease one or more of the individual peak emission intensities so that all peaks are at about the same intensity by reducing the microsphere concentration, moving the excitation to a shorter, less-optimal wavelength, or narrowing the slit width.

- Different lots of solvent may contain impurities that can degrade some of the fluorescent colors over time. We suggest making a single test solution containing solvent and all the fluorescent colors to be used. This solution should be read daily for as long as you normally allow your samples to remain in the solvent before reading them in the fluorimeter (e.g., if samples are to remain in solvent for 72 hours, read the test solution every day for 3 days). Repeat this with each new solvent lot. If degradation occurs in specific colors, avoid these colors or use a new solvent lot. We have observed that the stability of fluorescent colors vary between different lots of Cellosolve® acetate.

- Whenever possible, tissue and blood samples should be digested in glass containers. This decreases microsphere loss due to electrostatic attractions between the microspheres and plastic vials.