Protocol for use of Fluorescent Latex Microspheres

Modified from: Katz, L.C., Burkhalter, A., and Dreyer, W.J. (1984) Nature 310: 498-500.

This protocol is a modification of the protocol that was provided with the "Fluorescent latex microspheres" at the time of purchase.

This sheet summarizes most of the procedures for using fluorescent latex microspheres, or "beads," as a retrograde neuronal tracer. Further details are presented in Katz, L.C., Burkhalter, A., and Dreyer, W.D. Fluorescent latex microspheres as a retrograde neuronal marker for *in vivo* and *in vitro* studies of visual cortex. *Nature* 310: 498-500 (1984). The microspheres can be obtained from the following company: Lumafluor Corp., (lumafluor@aol.com): http://www.lumafluor.com/.

Supplied As:

The enclosed vial contains a concentrated solution of beads suspended in distilled water. For retrograde tracing of neuronal pathways, the solution can be used as is, or diluted. A dilution of 1:4 has been used without reducing the quality or extent of retrograde labeling and is recommended. In addition to distilled water, standard salt solutions (NaCl, KCl) can also be used as diluents. However, distilled water seems to work quite well.

Storage:

The bead solution should be stored in a humidified container, in a refrigerator. to prevent evaporation. The solution should **not** be frozen. Shelf life not established, but expected to be many years.

Application:

Injection by pressurized air system through a glass micropipet has been used successfully in the past. The beads will remain very localized to the injection site. For retrograde labeling of the retinotectal projections of *Xenopus laevis* tadpoles (i.e. St 45) it is not recommended to exceed 5-6 injections (using a pressurized air system set at 10 psi for 30msec per injection) per site.

The conditions for iontophoretic application of beads have not been worked out. The beads do apparently have a net negative charge.

Use electrode glass with capillary filament for the electrodes. Pull the electrodes in the conventional fashion. Place the back, open end of the microelectrode into the microsphere solution, and let the tip of the electrode fill by capillary action. You should actually see the solution filling the tip.

Survival Time:

The minimum effective post-injection survival time in most systems is about 24 hours, with labeling intensity increasing with longer survival, up to 48 hours. After 48 hours, no increase (or decrease) in labeling intensity is observed. Maximum survival time is not established, but labeling is unchanged in either intensity or extent even after 12 week survival times. Cells are probably permanently marked. No toxic effects on either animals or neurons have been observed.

It has been found in *Xenopus* that labeling is optimal after allowing 12-48 hours of post-injection survival (depending on the age of the animal).

Fixation and Processing:

Standard fixation procedures can be used. Rinse tissues/animals in a 0.1 M phosphate buffer wash followed by fixation in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Fix for 2 - 48 hours depending on the size of the tissue/animals. Preparations may be frozen sectioned on a cryostat using conventional procedures. Frozen sections may be collected, mounted on glass slides, and air dried. After drying, slides should be rinsed in phosphate buffer saline (PBS) coverslipped using Gel Mount (or other non-fluorescing mounting media) and allowed to harden before viewing. Use fingernail polish to seal the edges of the coverglass to prevent desiccation.

Attempts at clearing the slides in xylene after an alcohol dehydration series were unsuccessful and are not recommended. Keeping the slides in the dark will decrease fading of the labeled cells. Cells should remain good for at least one year.

Thus far, attempts to retain bead labeling after plastic embedding have not been successful.

Observation:

The dye in the microspheres is rhodamine; thus any fluorescence filter set for rhodamine can be used. Beads do not usually fade appreciably even after long periods of observation or photomicrography. However, some lightly labeled cells may experience photobleaching in some instances. Slides should be stored in the dark at 4°C.