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Fluoro-Gold Protocol and Use Guide

1. Background

The use of Fluoro-Gold is essentially the same as other fluorescent tracers. The main difference is that Fluoro-Gold is more flexible in terms of post-injection survival times, concentration range, tissue treatment, and compatibility with other histochemical techniques.

2. Storage and Shelf Life

Dry Fluoro-Gold should be kept in a light tight closed container at 4 degrees Celsius. Stored properly, Fluoro-Gold should have a shelf life exceeding one year. The dye in solution should also be kept in a light tight closed container at 4 degrees Celsius, in which case it will remain stable for at least six months.

3. Vehicle

Fluoro-Gold can be dissolved in distilled water or 0.9% saline, or utilized as a suspension in 0.2M neutral phosphate buffer.

4. Dye Concentration

Fluoro-Gold has been successfully used at concentrations ranging from 1-10%. Initially, a 4% concentration is advised. If undesirable necrosis occurs at the injection site, or labeling is too intense, reduce the concentration to a 2% solution. If you need to use more precise measurements, the molecular weight of Fluoro-Gold is 532.6 Daltons.

5. Dye Administration

A. Pressure Injection - This is probably the most frequently used mode of application. Volumes injected range from .05-1 μ l, typically .1-.2 μ l.

B. Iontophoresis - Discrete, small injection sites result from 4-10 second pulsed iontophoretic (+5 to +10ua/10min) application.

C. Crystal - A crystal of the tracer can be administered from the tip of a micropipette.

6. Post-Operative Survival Period

Good retrograde labeling has been observed with periods ranging from two days to two months. Survival periods of three to five days are typical. Long survival periods enhance filling of distal processes without diffusion of the dye from the cell.

7. Fixation

Almost any fixative, or no fixative, can be used; Phosphate neutral buffered saline containing 4% formaldehyde is frequently employed. Fixatives containing high concentrations of heavy metals (e.g. osmium, mercury) will quench the fluorescence, while high concentrations (over 1%) of glutaraldehyde may increase background fluorescence

8. Histochemical Processing

Tissue containing Fluoro-Gold may be processed according to virtually any common histological technique. This includes cryostat sections of unfixed tissue (10 μ m), frozen sections of fixed tissue (20 μ m), and thin sections cut from tissue imbedded in either plastic (.2-4 μ m) or paraffin (3-10 μ m). Frozen sections of fixed tissue are most frequently used.

9. Combined Methods

At this point of processing, sections may be further processed for a second marker such as autoradiography, HRP histochemistry, immunocytochemistry, a second fluorescent tracer, fluorescent counterstain, etc.

10. Mounting, Clearing and Coverslipping

Sections are typically mounted on gelatin-coated slides, air-dried, immersed in xylene, and coverslipped with nonfluorescent DPX plastic mounting media. Sections may be dehydrated with graded alcohols, unless this is not compatible with a second tracer. If Fluoro-Gold is to be combined with fluorescence immunocytochemistry, then sections are air-dried and directly coverslipped with neutral buffered glycerine (1:2).

11. Examination and Photography

Fluoro-Gold can be visualized with a fluorescence microscope using a wide band ultraviolet excitation filter. A gold color is emitted when tissue has been processed with neutral pH buffer, whereas a blue color is emitted when tissue is processed with acidic (e.g. pH 3.3) pH buffer. It can be photographed digitally or with film (use Ektachrome 200-400 ASA film for color prints and comparable speed film for black and white prints, for example Tri-X). Most exposure times range from 10-60 second exposures, depending on the objective magnification and the intensity of the label. Thirty (30) second exposures are about average. Multiple exposures may be exploited to simultaneously visualize Fluoro-Gold and another tracer. Thus, UV would be combined with bright field illumination to simultaneously locate Fluoro-Gold with HRP or silver grains in autoradiography. Similarly, blue light excitation can be combined to also visualize the green emission color of FITC, while green excitation light may be used to simultaneously observe the red emission color of propidium iodide, or ethidium bromide (a fluorescent counterstain).

Additional Information Concerning the Use of Fluoro-Gold

Vehicle

For pressure injections through a microsyringe or micropipette, Fluoro-Gold should be dissolved in distilled water or .9% saline. Fluoro-Gold may also be utilized as a suspension in .2M neutral phosphate buffer, however, the suspended particles may clog a fine micropipette tip so distilled water or .9% saline is the preferred vehicle. For iontophoresis, a 1% Fluoro-Gold solution is made up in .1M acetate buffer (pH=3.3). Well-cleaned (95% ETOH, water) glass micropipettes should have tips of 10-20 μ m. Optimal iontophoresis parameters are +1 to +5u amps delivered with pulsed current (4-10 seconds on, 4-10 seconds off) over a 10-20 minute period.

Injection Sites

Virtually any central or peripheral nervous system structure can be injected with Fluoro-Gold for analysis of retrograde transport. In the peripheral nervous system, ganglia and peripheral targets can be studied. For studies of peripheral nerve, the nerve should be cut or damaged and either dipped in, or injected with, aq. 5% solution of Fluoro-Gold. Since Fluoro-Gold is not significantly taken up by intact fibers of passage, the fibers must be cut or severely damaged for uptake of the dye to occur.

Transport and Survival Time

Fluoro-Gold is used as a retrograde axonal tracer, although orthograde axonal transport does occur. The survival time should be varied (especially to very short survival times of 12 hours - 2 days) to maximize orthograde transport in the specific neuronal system under study. For retrograde transport, the survival times should be varied from 4 days to 14 days. Seven to 10 days works for most systems, although long pathways (e.g., spinal cord to brainstem) and pathways in large mammals (e.g., cats, monkeys) may require longer survival times (e.g., 14 days). In addition, since Fluoro-Gold remains fast within retrograde labeled neurons, survival times of several months will also produce excellent results. For iontophoresis, a 2-5 day survival time is recommended. It is estimated that transport occurs at about 2 cm per day for mammals; it is slower for cold-blooded animals.

Tissue Processing

Tissue processing is covered in detail in the use guide and in the original publication (*Schmued and Fallon, 1986, Brain Research 377:147-154*). Since Fluoro-Gold is stable in many solvents and remains fast within retrograde labeled neurons, its use is compatible with many histochemical techniques. It can be used with other retrograde tracers, immunofluorescence, PAP and ABC immunocytochemistry, HRP histochemistry, autoradiography, counterstains (ethidium bromide is the preferred fluorescent counterstain), paraffin embedding and plastic embedding. However, if tissue is unfixed, additional processing of tissue in aqueous solutions for over an hour or two will result in loss of Fluoro-Gold fluorescence from labeled neurons. Fluoro-Gold may be useful in electron microscopy. Fluoro-Gold can be used in a brain, which has been sectioned and transferred to phosphate buffer. Sections are typically mounted on gelatin-coated slides, air dried, immersed in xylene and coverslipped with DPX plastic mounting media. Tissue may also be viewed on slides without further processing, can be run through graded alcohols for dehydration, or, for immunocytochemistry, the sections can be air dried and directly coverslipped with neutral buffered glycerine (1:2).

Examination and Photography

Fluoro-Gold is visualized with a fluorescence microscope using a wide band ultraviolet (UV) excitation filter. Use the same filter pack you would for other fluorescent retrograde tracers excited under wide band UV (e.g., True Blue, Fast Blue, Nuclear Yellow), such as the Leitz Phloem filter system A (Wide Band UV, Excitation filter BP 340-380), Mirror RKP 400, Barrier Filter LP 430). Objectives should be made especially for fluorescence microscopy (such as that made by Zeiss) glycerine, or water. Since plastic does absorb UV light, it is not advised to view through plastic Petri dishes, etc. Recommended films are T-Max (Kodak, black & white) and Ektachrome 200 Kodak, color slides). Exposure times usually vary from 20 seconds to 1.5 minutes.

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Antibody to Fluoro-Gold Protocol and Product Information

Storage and Preparation of the Antibody

You will receive the Antibody to Fluoro-Gold in a small vial. It is a polyclonal antibody raised in rabbit. The vial will contain approximately 100 μ l of the antibody solution. You will not receive the Antibody frozen, but it should be immediately frozen and stored in a cold (-20° C), dark, dry environment. Once the solution is thawed to prepare your working titer, do not refreeze the solution. The freezer section, without the frost-free feature, of a good commercial refrigerator should suffice. If properly and continuously frozen, the antibody solution can be stored up to one year.

The antibody solution should remain frozen until ready for use. The 100 μ l aliquot of the Antibody is at a dilution of 1/100. The Antibody can be further diluted 1/50,000 to 1/100,000 times in BSA diluent (50 mM KPBS, 0.4% Triton, 1% BSA, 1% NGS) to produce 50ml to 100ml of working titer. This means you can dilute the solution you receive by 500 to 1000 times. This should treat 300 to 1000 sections if you use the Vector elite kit. After preparation, the antibody solution can be stored for up to seven days in a cool (4° C), dark and dry environment. The refrigerator section of a good commercial refrigerator should suffice. We do not recommend that the solution be used beyond seven days after preparation.

Use of the Antibody

Fluoro-Gold can be injected using several different methods, including pressure, iontophoretic and other applications developed by a variety of researchers. See *Schmued and Fallon, Fluoro-Gold: "A fluorescent retrograde axonal tracer with numerous unique properties"*, *Brain Research*, 377 (1986) 147-154 as well as *Pieribone and Aston-Jones, "The Iontophoretic Application of Fluoro-Gold for the study of afferents to deep brain nuclei"*, *Brain Research*, 475 (1988) 259-271. Many researchers have developed their own modified procedures. Use of the antibody should not be dependent upon the methodology used to employ Fluoro-Gold.

After the Fluoro-Gold has been injected, floating sections (we used thirty μ m sections from a rat perfused with 4% formaldehyde) are incubated with the Fluoro-Gold Antibody solution overnight at 4° C. Sections are washed, then incubated in Biotinylated GAR (Vector Labs) at 1/1000 for 1 hour at room temperature and washed again. Sections are then incubated in Avidin/Biotin (Vector Labs) at 1/1000 for 1 hour, washed and transferred to Diaminobenzidine (.04%) and Nickel Chloride (2.5%) in 0.1 M NaAcetate with 0.06% H₂O₂ for six minutes. Sections are then washed, mounted, dried, dehydrated and cover slipped.

It has been our experience that if stored and prepared in the manner set out above, each vial of the antibody should treat 300 to 1000 thirty μ m sections of the albino rat brain (or similar sized animals) if you use the Vector elite kit. However, you should experiment with the concentration and procedures to determine which best fit your circumstance.