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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.