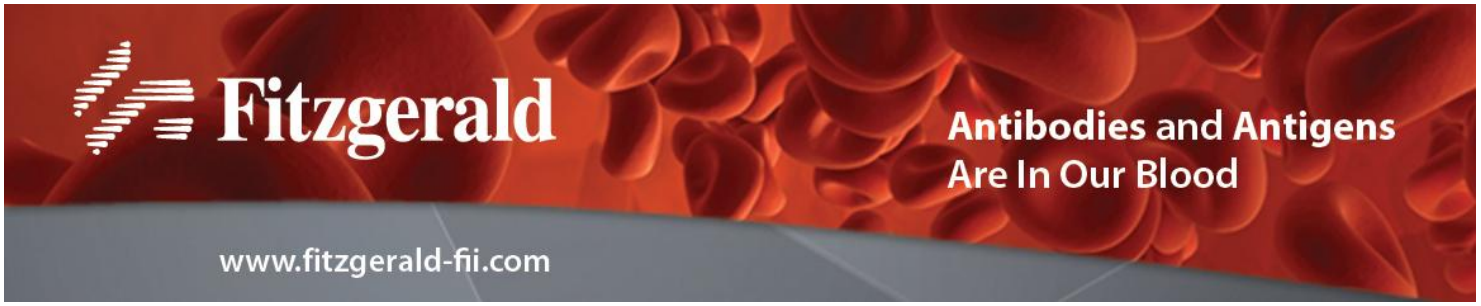

Guidelines for Colloidal Gold Assays

How to choose a Gold Conjugate

The aim of an antibody incubation with a specimen, whether it be a section, cells in suspension, a tissue slice, a blotted membrane or a rapid diagnostic test assay, is to achieve the most intense specific signal and the least non specific background possible.

Direct or Indirect labelling (antibodies)

Gold conjugates may be used directly or indirectly to label antigens as indicated above.

- In the direct method, the gold is conjugated to the primary antibody, whether monoclonal or polyclonal. This allows a single incubation to be performed and provides the simplest detection system.
- In the indirect method a primary unlabelled antibody is applied to the specimen to locate the antigen. This is followed by a gold labelled secondary that detects the primary antibody. This gold labelled secondary antibody is almost always an affinity purified polyclonal. In this way, an amplification of the signal is achieved, often up to 10 times greater compared with a direct incubation, according to the particle size.
- The choice of the most appropriate system depends upon the type of component to be detected and what binding proteins are available. Some guidelines on making this choice are given below.

An extension of the indirect method may be used where there is no appropriate gold labelled secondary to match the primary antibody. For example, if the unlabelled primary is from Pig and there is no matching gold labelled anti-Pig, an intermediate secondary antibody such as Rabbit anti-Pig may be used as a second step, followed by a gold labelled third antibody such as Goat anti-Rabbit, that will detect the second antibody.

The indirect method is the most common for studying cells and tissues and avoids the need to label every primary antibody. It provides a universal method for detecting any primary antibody from the same primary species, ie all Rabbit antibodies may be detected by Goat anti-Rabbit gold conjugates, etc.

This table indicates directly labeled primary antibodies and proteins available from Fitzgerald:

62-L26B	Luteinizing Hormone alpha antibody (Gold Colloid)
62-H25C	hCG beta antibody (Gold Colloid)
62-L25A	Luteinizing Hormone beta antibody (Gold Colloid)
62-H05A	HBsAg antibody (Gold Colloid)
62-M25A	Methamphetamine antibody (Gold Colloid)
62-T43A	THC antibody (Gold Colloid)
64-IT77	p15, p17, p47 Treponema Pallidum protein (Gold Colloid)

This table indicates secondary gold labelled antibodies available from Fitzgerald:

43R-ID067GD	Donkey anti Chicken IgY (H + L) (12 nm Gold Colloid)
43R-ID059GD	Donkey anti Chicken IgY (H + L) (18 nm Gold Colloid)
43R-IG100GD	Goat anti Rabbit IgG (5 nm Gold Colloid)
43R-IG085GD	Goat anti Rabbit IgG (15 nm Gold Colloid)
43R-IG086GD	Goat anti Rabbit IgG (20 nm Gold Colloid)
43R-IG084GD	Goat anti Mouse IgG (10 nm Gold Colloid)
43R-IG087GD	Goat anti Mouse IgG (20 nm Gold Colloid)
43R-IG157GD	Goat anti Mouse IgG (10 nm Gold Colloid)

When to use F(ab') fragments?

In some applications background labelling may be a problem due to the attraction of the Fc region of the antibody-gold conjugate to tissue components (called Fc receptors). Normally this is blocked by the simple application of normal serum prior to the first antibody . If the problem persists, however, then gold labelled F(ab') fragments of antibodies may be used.

When to use antibodies, Protein A, Protein G or Protein A/G?

The table indicates Protein A and G conjugates available from Fitzgerald:

62R-A102GD	Protein A (40nm Gold Coloid)
62R-G100GD	Protein G (40nm Gold Coloid)

Protein A (PAG), Protein G (PGG) and Protein A/G(AG) are all separate proteins and will mimic a secondary antibody by binding to the Fc part of primary antibody. In some situations they may be used in place of the secondary antibody. Unlike secondary antibodies, however, it is believed that there is only a single binding site for these proteins on the primary IgG. They do not bind with great affinity to IgM or IgA molecules. The advantage of these proteins is that they provide a universal second step for a wide range of primary antibody species. They each bind with varying affinities to IgG molecules of different species as shown in the table (below). However, if possible we would always recommend the selection of a species specific secondary antibody in preference to Protein A,G or A/G.

Relative Affinities of Specific Ig Binding Proteins			
Primary Antibody	Protein A: gold (PAG)	Protein G: gold (PGG)	Protein A/G: gold (AG)
Rabbit IgG	+++	+++	+++
Mouse IgG	++	++	+++
Mouse IgM	+/-	+/-	+/-
Mouse IgA	+/-	+/-	+/-
Rat IgG	+/-	+	+++
Human IgG1	+++	+++	+++
Human IgG2	+++	+++	+++
Human IgG3	+/-	+++	+++
Human IgG4	+++	+++	+++
Human IgA	+	-	-
Human IgM	+	-	-
Guinea Pig IgG	++	++	++
Goat IgG	++	++	++
Bovine IgG	++	++	++
Sheep IgG	+/-	++	+++
Chicken IgG	+/-	+	+/-

Streptavidin or Goat anti-Biotin?

The table indicates Streptavidin conjugates available from Fitzgerald:

80R-S103GD	Streptavidin (5 nm Gold Colloid)
80R-S102GD	Streptavidin (15 nm Gold Colloid)
80R-S101GD	Streptavidin (40nm Gold Coloid)

For the indirect detection of a biotinylated primary antibody or nucleic acid probe there is a choice of Streptavidin: gold or Goat anti-Biotin: gold conjugates. Historically streptavidin has been the most frequently used detector for biotin because of the very high affinity constant between them. Most recently, however, Goat anti-Biotin has been shown to be a rather more sensitive detector of biotin compared to streptavidin when conjugates to gold particles. This is because of the relatively large molecular size of the anti-biotin molecule (160,000 daltons) compared to streptavidin (40,000 daltons) and the distance between the binding site of the gold on the Fc from the binding region of the antibody F(ab'). This is especially so when using larger gold particles but becomes insignificant for 5nm and 1nm gold conjugates. On occasions where background from non-specific attraction of goat antibodies may cause a problem, streptavidin may provide a cleaner result. We recommend beginning with Goat anti-Biotin gold conjugates for the detection of biotin.

Cationic gold

Cationic gold allows highly sensitive and discrete microscopical studies of anionic (ie negative) sites in cells and tissues (1). The gold conjugate is made by careful conjugation to poly-L-Lysine, a highly positive amino acid chain. A simple one step incubation of sections with the diluted Cationic Gold conjugate reveals subcellular sites having net negative charge. The charge distribution can be seen at a range of magnifications by using Cationic Gold of different particle sizes. Most cells of eukaryotic origin have a net negative surface charge from anionic plasma membrane components. This charge distribution is thought to be important in movement of various soluble

macromolecules across cell walls. Thus the role of the surface charge in cellular behavior through interaction with neighboring cells can be studied. For in vitro and in vivo studies Cationic Gold may also be used effectively to study the uptake of anionic material by endocytosis.

1. *Bush MS and Allt G(1990) "Blood nerve barrier: distribution of anionic sites on the endothelial plasma membrane and basal lamina." Brain Res 535, 181-188*

How to Choose the Gold Particle Size

The tables indicate different gold and silver conjugates and enhancement kits available from Fitzgerald:

62R-GC003	Gold Colloid (2 nm)
62R-GC007	Gold Colloid (5 nm)
62R-GC010	Gold Colloid (10 nm)
62R-GC002	Gold Colloid (15 nm)
62R-GC005	Gold Colloid (20 nm)
62R-GC006	Gold Colloid (40 nm)
62R-GC008	Gold Colloid (50 nm)
62R-GC009	Gold Colloid (60 nm)
62R-GC011	Gold Colloid (100 nm)
62R-GC012	Gold Colloid (200 nm)
63R-SC001	Silver Colloid (20nm)

55R-SEKL15	Silver Enhancing kit
55R-SEKB250	Silver Enhancing kit
55R-PRO500	Gold Staining Kit

A wide range of particle sizes can be conjugated to proteins and macromolecules. In principle, smaller gold particles produce a higher labelling intensity on the specimen. This is because of the reduced stearic hindrance to antigen detection. Typically an antibody of 160,000 daltons molecular weight will have a linear dimension of 8nm. Thus a 1nm gold particle,attached to the Fc region will hardly impede the antibody activity. A 20nm particle, however, while being more visible, will produce a greater stearic hindrance by its proximity to the antigen binding region of the antibody. In addition the increased charge repulsion between larger particles reduces the number of labelled antibodies gaining access to the target antigen.

Different particle sizes are appropriate for different types of application as described below.

a) **Light Microscopy**

Gold particles cannot easily be seen in the light microscope by bright field viewing. They must thus be silver enhanced for greatest visibility. Small gold particles will give the greatest number of gold labelling on the specimen and each particle can then be subsequently silver enhanced for maximum visibility of signal. A choice can be made between 1nm and 5nm gold conjugates.
The 5nm conjugates are used for most standard purposes and are recommended for initial studies. For even greater labelling intensity the 1nm gold conjugates are preferred. In both cases the gold signal is silver enhanced to make it visible in the light microscope. The 1nm gold conjugates are particularly useful when penetration into cells and tissues (eg preembedding labelling) is required.

b) **Transmission Electron Microscopy**

For electron microscopy any particle size may be employed. For low magnification work larger particles (eg 15-30nm) are more easily seen. For high magnification studies the smaller particles (eg 5-10nm) are preferred. Again, smaller particles give higher labelling

intensity and may be subsequently silver enhanced on the section to produce larger particles with this high labelling intensity. For ultra high sensitivity the 1nm gold particles can also be chosen but are usually visualized only after silver enhancing on the section.

For those just beginning immunogold labelling in the EM or performing studies over a range of magnifications, 10nm gold conjugates are recommended.

c) **Scanning Electron Microscopy**

The resolution of the scanning electron microscope indicates that larger gold particles (eg >20nm) should be used for detection by back scattering electron signals. However, as mentioned above, a greater intensity of labelling is achieved with smaller gold particles which can be subsequently silver enhanced with the LM/EM Silver Enhancing Kit (55R-SEKL15). We would therefore recommend that a choice is made for SEM studies between 20 or 30nm gold conjugates for direct (unenhanced) viewing, or 5nm conjugates which can be simply silver enhanced within minutes for observation at low magnifications. With the latter approach each gold particle grows spherically to approximately 3-nm in 3-5 minutes. The reaction is simply stopped by washing in water.

d) **Blotting**

For blotting applications where the gold conjugates are to be applied to proteins immobilized on nitrocellulose membranes, the choice is between 1nm and 20nm BL Grade gold conjugates. Even 5nm conjugates may also be used very effectively (LM Grade) together with subsequent silver enhancing. For ultra high sensitivity 1nm conjugates are preferred in combination with silver enhancing. However the 20nm gold conjugates allow blotted proteins to be identified on the immobilizing membrane without silver enhancing. The 1nm and 5nm (LM Grade) gold conjugates,because of their much reduced stearic hindrance and greater particle density in solution, may be diluted much further to provide the same final intensity compared with the 20nm conjugates. The 1nm and 5nm (LM) gold particles must be enhanced with the BlottingSilver Enhancing Kit (55R-SEKB250).

Glycerol or Non Glycerol?

Standard Gold Conjugates are presented in a buffer containing 20% glycerol to allow good freezing characteristics. In most cases this causes no difficulties since the gold conjugates may be used highly diluted and specimens are fixed and embedded. On some occasions, however, when using the gold conjugate at high concentrations (eg 1:2) the presence of high glycerol content may interfere with satisfactory labelling of cells. Such specimens may include fixed cells in suspension or in cell culture where the glycerol may cause shrinkage of the cells. Such high concentrations of glycerol may also cause a lack of clarity of the gold particles in the EM and may even slow down the rate of labeling through increased viscosity. For such applications it is recommended that gold conjugates without glycerol are used.