TAAB Gold Probes

Gold probes have, since their introduction in 1971, become widely used in both light and electron microscopy for the identification of proteins and antigens in cells and tissues. The technique has enjoyed enormous growth over the last few years with a vast growth in the number of applications in animal and plant biology and microbiology. In addition the sensitivity and specificity of the technique has established it as an important tool in immunoblotting for the study of proteins (Western blots) and DNA fragments (Southern blotting). Gold probes are stable, sensitive, non-hazardous, extremely economical and easy to use. TAAB gold conjugates may be stored for *12 months at 4°C or longer if frozen at –25^{0}C or below* to give long term reproducible results. A pack of microtubes is available for customers wishing to aliquot and freeze their conjugates on delivery.

All our gold conjugated antibodies are affinity purified to ensure low cross reactivity. EM grade antibody conjugates have at least 85% singlets. Each product is provided with a quality assurance certificate indicating the sensitivity, concentration, exact particle size and coefficient of variation, and freedom from clustering.

GOLD CONJUGATES
Choice of GOLD Conjugates:

Electron microscope (EM) grade conjugates are available as proteins linked to 1, 5, 10, 15 and 20nm gold particles, with 30 and 40nm gold particles available for certain products, all non-overlapping and allowing multiple labelling to be achieved for several antigens on the same specimen. **Which Particle Size?** - at magnifications above 50,000x the 5 and 10nm sizes are recommended. For lower magnifications, the 15, 20, 30 or 40nm sizes should be used. *Those users just beginning immunogold labelling are recommended to use 10nm particle size.* For 1nm and 5nm gold conjugates, a combination of gold labelling with silver enhancing will yield larger size particles with high labelling intensity.

Light microscopy (LM) grade conjugates are of 1nm and 5nm gold particles to provide maximum penetration into sections. In either case the particles are not immediately visible in the LM. This is because the resolution of the LM is >200nm. With silver enhancing (Silver Enhancing Kit) you can grow the particles within minutes to almost perfect spheres of a size large enough to be seen at high intensity in the LM. Gold particles are inert and will not change with time. Silver enhancing stains give a permanent intense brown/black signal.
All common counterstains may be used on tissues after labelling with gold conjugates. Enzyme based labels may be used in conjunction with gold labels for multiple staining of antigens on cells and tissue. **Which Particle Size?** – for most purposes 5nm gold conjugates are suitable. Where higher labelling intensity is required or where penetration through cell membranes is necessary the 1nm gold conjugate should be selected. The 1nm gold conjugate may be diluted much further for use compared with the 5nm gold conjugate. In both cases the Silver Enhancing Kit is used to increase the signal.

Blotted Proteins (BL) grade is provided as 1nm and 20nm particle sizes for optimum visibility and silver intensification. Blotting applications of gold conjugates include the demonstration of macromolecules, antigens, antibodies, and other proteins immobilised by a suitable negatively charged membrane such as nitrocellulose. **Which Particle Size?** – due to the high visibility of 20nm gold particles accumulating on the membrane, a strong signal is obtained after incubation without the immediate need for further silver enhancing. Nevertheless, silver enhancing of the gold stain will increase the sensitivity by 10 – 100x. For further sensitivity 1nm gold particles may be used. These conjugates produce a very high labelling intensity that is converted to an intense black stain with further silver enhancement.

Protein A , Protein G or Protein A/G conjugates offer the advantage of being universal secondary labelling reagents for most primary IgG antibodies. Individual gold labelled secondary antibodies, however, offer higher sensitivity through multiple binding to the primary antibody.

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Streptavidin or Goat anti-Biotin conjugates have a very high affinity for biotin. They provide a sensitive and specific method for the detection of biotinylated primary antibodies, proteins or DNA in both microscopical and blotting applications. Goat anti-Biotin has been shown to be a rather more sensitive detector of biotin compared with streptavidin when conjugated 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').

Cationic gold allows highly sensitive and discrete microscopical studies of anionic (i.e. negative) sites in cells and tissues. The gold conjugate is made by careful conjugation to Poly-L-Lysine, a highly positive amino acid chain.

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.

Lectin gold conjugates Lectins are carbohydrate binding proteins of non immune origin which agglutinate cells and precipitate glycoconjugates.

CODE: **(H) =** Heavy Chain Specific **(H+L) =** Heavy + Light Chain Specific **(AH) =** Absorbed with Human Serum Proteins **(Rat Abs)** Absorbed against rat serum proteins **(Mouse Abs)** Absorbed against mouse serum proteins

UNCONJUGATED COLLOIDAL GOLD

TAAB Gold Colloids are supplied ready for conjugation to proteins, antibodies, or many other types of macromolecule for binding and reaction labelling studies. They are shipped in sterile containers as 100ml or 500ml volumes. They are available as different particle sizes for EM, LM or Blotting applications. The colloids may be stored for *at least 12 months at 4°C* if left unopened. ***Do not freeze***. Each colloid is provided with a quality control certificate.

Blocking Reagents

Non specific labelling can occur on specimens during immunolabelling procedures. The source of this background labelling must be determined by the careful and systematic use of controls and eliminated for the proper analysis of the specimen. Background labelling can occur from a number of sources, either in the specimen or in the incubating solutions. In either case the background can be substantially reduced by the careful use of blocking reagents.