Gold Conjugates

Technical Information and Guidelines for use in Immunolabeling

The enclosed gold conjugate is made to the highest standards and specifications to yield excellent results when correctly used. The guidelines are straightforward and intended for use with either light or electron microscopy immunolabeling applications. All gold conjugates are supplied in one of the two buffers listed below. Buffer constituents dictate the shelf life and storage conditions for the individual conjugates.

Electron microscopy (EM), light microscopy (LM) and blotting grade (BL) conjugates are supplied in the following buffer: 20mM Tris (tris-hydroxymethyl-aminomethane); 20mM sodium azide; 154mM NaCl; 1% BSA; 20% glycerol; pH 8.2. Recipe to make 100ml: 0.242g (20mM) Tris + 0.9g (154mM) NaCl + ultrapure water to make 100ml. Adjust pH from 7.2 to 8.2 with 1N HCl or 1N NaOH. **Storage:** Stable for 1 year at 4ºC; stability for 2+ years at –20ºC. The conjugates demonstrate remarkable stability at ambient temperatures for up to 7 days. Repeated freezing and thawing is not recommended.

Ultra small (1nm) gold conjugates are supplied in the following buffer: 10mM Na_2HPO_4; 3mM KH_2PO_4; 123mM NaCl; 20mM sodium azide; pH 7.2. Recipe to make 100ml: 0.148g Na_2HPO_4 + 0.043g KH_2PO_4 + 0.72g NaCl + ultrapure water to make 100ml. Adjust pH from 7.2 to 8.2 with 1N HCl or 1N NaOH. **Storage:** Stable for 1 year at 4ºC – **DO NOT FREEZE**. The conjugates demonstrate remarkable stability at ambient temperatures for up to 7 days.

Gold Conjugate Technical Information

Conjugate	Gold Particle Size	O.D.	Protein Concentration µg/ml	Gold Particles/ml	Proteins/Particle
Immunoglobulin	1nm	-	50	2.0×10^{15}	1 to 3
Streptavidin	1nm	-	25	2.0×10^{15}	1 to 2
Immunoglobulin	5nm	3.0	36	1.7×10^{14}	3
Protein A	5nm	3.0	12	1.7×10^{14}	4
Streptavidin	5nm	3.0	20	1.7×10^{14}	5
poly-L-lysine	5nm	3.0	100	1.7×10^{14}	Unknown
Immunoglobulin	10nm	3.0	30	1.7×10^{13}	12
Protein A	10nm	3.0	10	1.7×10^{13}	16
Streptavidin	10nm	3.0	20	1.7×10^{13}	20
poly-L-lysine	10nm	3.0	60	1.7×10^{13}	Unknown
Immunoglobulin	15nm	4.0	30	5.0×10^{12}	27
Protein A	15nm	4.0	10	5.0×10^{12}	36
Streptavidin	15nm	4.0	20	5.0×10^{12}	45
poly-L-lysine	15nm	4.0	60	5.0×10^{12}	Unknown
Immunoglobulin	20nm	4.0	30	2.0×10^{12}	48
Protein A	20nm	4.0	10	2.0×10^{12}	64
Streptavidin	20nm	4.0	20	2.0×10^{12}	80
poly-L-lysine	20nm	4.0	50	2.0×10^{12}	Unknown
Immunoglobulin	30nm	5.0	15	8.0×10^{11}	86
Immunoglobulin	40nm	5.0	10	4.5×10^{11}	150

Product Information

Each gold conjugate has a technical data sheet which indicates the following information: 1) Number of particles counted; 2) Mean particle diameter; 3) Coefficient of variation given as a percent; 4) Percent of single particles; 5) Percent of particles larger than triplets; 6) Minimum detectable protein. The coefficient of variation is an important parameter in describing the relative distribution of gold particle sizes around the mean for a given batch. The coefficent of variation equals the standard deviation divided by the mean.

Normal Gaussian distributions work as follows: ±1 standard deviation describes 68% of the area under the curve; ±2 standard deviations describes 95% of the area under the curve; ±3 standard deviations describes 99.73% of the area under the curve. As an example, you have purchased a gold conjugate - Goat anti-Rabbit IgG (H+L), 10nm - having a mean particle diameter of 9.8nm

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with a **coefficient of variation of 4.1%.** First, the standard deviation needs to be determined. In this case it is 0.402nm (4.1% x mean particle diameter). Statistically 68% of the particles will be from 9.40 to 10.20nm, 95% from 9.00 to 10.60nm and 99.73% from 8.60 to 11.00nm. A reliable size characterization has been determined for the batch.

Sample Fixation

All antigens and tissues react differently to different fixatives and preservation strategies (e.g. high pressure freezing, cryoultramicrotomy, etc.). As a result no one method will produce results all the time. Fixation is a very important step in determining the success of most immunolabeling procedures. Paraformaldehyde based fixatives are most often used in concentrations from 0.5% up to 4% or higher. Often glutaraldehyde is added in concentrations typically from 0.1 to 0.5%. Cacodylate and the phosphate (Millonig, Sörensen) buffers are popular and the zwitterionic buffers such as HEPES or PIPES can be used as well for aldehyde fixation. The quenching of unreacted aldehydes is recommended by one of the following methods: 1) 0.05-0.1M ammonium chloride in buffer at 4ºC for a few hours; 2) 0.1-0.2M glycine in buffer for a few hours; 3) sodium borohydride (0.1 mg/ml) in buffer for a few hours. Osmium tetroxide is not often used for tissues processed for immunolabeling. Berryman and Rodewald, 1990, J. Histochem. Cytochem., 38:159-170 describe an excellent method to attain membrane contrast without the use of osmium.

Resin Infiltration/Polymerization

The hydrophilic polar resins (Lowicryl K4M and K11M; LR White; UNICRYL™; LR Gold) have proven to be the best based on immunoreactivity. Cryoultramicrotomy methods are more difficult but yield excellent results when done correctly. The polar resins are partially water soluble and do not need to be dehydrated to 100%. Apolar hydrophobic resins (Lowicryl HM20 and HM23) are available for use as well. The epoxy resins, for most applications, are not recommended. Dehydration, infiltration and polymerization with polar and apolar resins can be done at room temperatures or in the cold (progressively lower temperature techniques). Polymerization of polar and apolar resins is done by UV for immuno applications; however, UV as well as oven cures can be done with UNICRYL™ and LR White.

Positive/Negative Controls

Positive as well as negative controls should be a part of all immunolabeling studies. Reagent and system dynamics require that controls be used to identify optimum signal-to-noise ratios (i.e. correct dilution of primary and secondary antibodies), viability of antibodies being used and necessary blocking steps or strategies. Tween 20® (Prod. No. 15713) , Fish Gelatin (45%) (Prod. No. 15717) and BSA (Prod. No. 15716) are good generic blocking reagents that can be mixed with antibody or rinse solutions. Normal serum from donkey (Prod. No. 15714-D), fetal calf (Prod. No. 15714-FC), mouse (Prod. No. 15714-M), sheep (Prod. No. 15714-S), goat (Prod. No. 15714) or rabbit (Prod. No. 15712) can be added to improve labeling specificity and reduce noise.

Sample Labeling Protocol

Immunolabeling should be done in a humid chamber to protect against drying.

1. Blocking step: 20-30 minutes at room temperature (recommend 0.1% Tween 20® and 0.1% Fish Gelatin to start – add BSA 1-5% and the appropriate normal serum to reduce background if present).
2. Primary Antibody Incubation: 30-60 minutes at room temperature or 37ºC.
3. Buffer Rinse: 30 minutes at room temperature with a buffer change every 5 minutes.
4. Gold Conjugate Incubation: 30-45 minutes in duration at room temperature or 37ºC.
5. Buffer Rinse: A method found beneficial to reduce background is to elevate the NaCl concentration to approximately 2.5M from 1.54mM. The change in molarity has a beneficial effect on background due to ionic interactions of the gold conjugate (net negative change) and the substrate. Rinse for approximately 10 minutes and switch to water (ultrapure) for at least four changes of five minutes each.
6. Post Stain: 2% uranyl acetate (aqueous or alcoholic) for 5 minutes, rinse well in ultrapure water and then stain for 5 minutes with lead (25ml DI water, dissolve one sodium hydroxide pellet and add 0.125g of lead citrate). Rinse well after lead staining.

NOTE: Elevated temperatures (i.e. 37ºC) during incubations require less time. Less time helps to keep the sections on the grids. Nickel or gold grids should be used. All grids, regardless, should be cleaned prior to use (1N HCl – 100% ETOH – 100% Acetone – Dry in 60ºC oven on filter paper)

Technical Support

Contact Ted Pella, Inc. if you have any questions. Ask for the technical product specialist by using the phone, Email or FAX numbers shown on this Technical Note. We are happy to help you.

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