Center for Cell Imaging Department of Cell Biology

Contents

Preparation of Colloidal Gold Conjugates.

Colloidal gold has been used for centuries in the preparation of stained glass for windows and fine glassware. In recent years, colloidal gold particles have become a useful tool for microscopists. Colloidal gold particles are especially useful for biological electron microscopy. Some of the reasons why are listed below.

- Homogeneous preparations of particles varying in size from 3 µm to 20 µm can be easily prepared.
- Colloidal gold suspensions are inexpensive to prepare.
- Most proteins can be easily coupled to colloidal gold particles.
- Proteins coupled to gold particles do not appear to lose their biological activity.
- The colloidal gold particles can be easily seen in the electron microscope.
- Colloidal gold probes can be used for light microscopy. The larger gold particles can be directly observed by the light microscope. Smaller particles are detected by silver enhancement or epipolarized illumination.
- The same probes can be used for both LM and TEM immunocytochemistry.

Preparation procedure for producing gold sols

This page will explain a simple, published protocol for preparing colloidal gold particles, how to couple these particles to protein A and how to purify the probes after they have been made.

To make 100 ml of gold sol, two stock solutions have to be prepared.

Solution A

80 ml distilled water and 1 ml 1% aqueous gold chloride.

Solution B

4 ml 1% tri-sodium citrate.2H2O + 16 ml H2O + variable amount of 1% tannic acid [Mallinckrodt #1764] (see table 1 below).

When 1 ml or more tannic acid is needed, add an equal amount of 25mM

potassium carbonate for pH adjustment.
Warm up solutions A and B to 60˚C and mix them while stirring. When the red color has formed heat up to 95˚C and cool the solution on ice. The larger particles (where lower concentrations of tannic acid are used) take longer to form and the red color can take up to 1 hr to develop.

Coupling the Protein A to the Gold Particles.

A gold sol will bind proteins more efficiently when the pH of the solution is close to the pI of the protein (for protein A the pI is pH 5.1). A tighter binding occurs at higher pH but this may have a denaturing effect on the protein making the probe less effective. Having too much protein coupled to the gold particles may also be disadvantageous: some of the weakly bound protein may detach from the particles. This will make the probe less effective because the free protein will compete for binding sites with the gold-labelled protein. Horrisberger and Clerc (1985, Labelling of colloidal gold with protein A. A quantitative study. Histochemistry, 82, 219-223) recommend binding the protein A to colloidal gold at pH 6.0.
Check the pH of the gold sol with pH paper (the gold sol will block a pH electrode) and adjust the pH with 0.1N sodium hydroxide.
Protein A (Boehringer Mannheim) is dissolved in distilled water at 1mg/ml. A microtitration assay will show the correct amount of protein A to add to the gold sol (between 4-6µg/ml).
Add the protein A while stirring the gold sol. After 5 min add 10% bovine serum albumin (BSA) in PBS to a final concentration of 0.2% (2ml/100ml) to maximally stabilize the sol.

Purification of the protein A-gold.

The protein A-gold is centrifuged in a Ti 70 rotor in a Beckman ultracentrifuge at the appropriate speed (see table 2) for 30 min at 4˚C. At the correct speed the gold particles will settle to the bottom of the tube as a loose pellet. Remove the supernatant without disturbing the pellet and re-suspend the loose part of the pellet in PBS containing 0.2% BSA.
A second centrifugation step down a gradient will remove any gold particles of the wrong size. This is done on a 10-30% continuous glycerol gradient at 4˚C. Layer 1-2 ml of protein A gold onto the top of the gradient, spin in an SW40 rotor at the appropriate speed (see table 3) for 45 min at 4˚C. The dark red band in the middle of the gradient is collected. All aggregated protein A gold particles will have been removed.

Storage.

Immunogold probes may lose activity within weeks, due to the dissociation of the

proteins from the gold particles.
Dialyse the protein A gold against 50% glycerol in PBS and store at -20°C or freeze down small aliquots in liquid nitrogen and store at -70°C.
To determine the concentration at which to use the protein A gold, measure the optical density (OD) at 520 nm of a 1:100 diluted solution in PBS. Use a dilution with an O.D. of between 0.05 and 0.1 where there is no significant background. If using a primary antibody then the optimal dilution of this first antibody must be known. Sections can be treated with protein A gold alone to determine the background labelling.

Table 1:

The influence of the tannic acid concentration, during gold sol formation, on the size of the gold particles.

Gold particle size	1% Tannic acid amount (for 100ml final sol)
3.5 nm	5.000 ml
4.0 nm	2.500 ml
5.0 nm	1.000 ml
6.0 nm	0.500 ml
7.5 nm	0.250 ml
9.5 nm	0.100 ml
10.0 nm	0.080 ml
11.5 nm	0.050 ml
14.0 nm	0.025 ml

When 1 ml or more of tannic acid is used an equal amount of 25 mM potassium carbonate must be added to neutralize solution B.

Table 2:

First centrifugation, using a Ti 70 rotor for 30 min at 4°C. This step will concentrate the colloidal gold probe in the bottom of the tube.

Gold particle size	Ti 70 rotor speed	g (r max)
4 nm	47000 rpm	225000
5 nm	45000 rpm	210000
6 nm	42000 rpm	185000
7 nm	38000 rpm	155000
8 nm	33000 rpm	105000
9 nm	30000 rpm	92000
10 nm	27000 rpm	75000
12 nm	18000 rpm	37000

14 nm	10000 rpm	7000

Table 3:

Second centrifugation using SW 40 rotor for 45 min at 4°C. This will separate the different sizes of gold particle along the gradient.

Gold particle size	SW 40 rotor speed	g (r max)
4 nm	40000 rpm	284000
5 nm	37000 rpm	240000
6 nm	32000 rpm	170000
7 nm	30000 rpm	150000
8 nm	26000 rpm	115000
9 nm	22000 rpm	85000
10 nm	19000 rpm	65000
12 nm	11000 rpm	20000
14 nm	5000 rpm	5000

The 4 nm gold must be centrifuged for 1 hr for best results.
The 5nm gold can also be centrifuged at 35000 rpm for 1 hr.

Coupling bovine serum albumin (BSA) to gold particles

BSA-gold is a useful marker for studying the endocytic processes in mammalian cells. Typically, the living cells are incubated in a BSA-gold suspension with a final OD, at 520 nm, of 4, meaning that a large amount of gold probe is needed for these experiments. The above protocol for preparing protein A-gold can be followed for preparing BSA-gold but BSA is substituted for the protein A. Usually, the stabilization requires more BSA than protein A (we use 24 ug/ml). The centrifugation values are the same.
After the gold probe has been concentrated and purified it is dyalized against PBS, or culture medium, before being added to the living cells.

Useful References

Slot, J. W. &H. J. Geuze 1981. Sizing of protein A-colliodal gold probes for immunoelectron microscopy. J. Cell Biol. 90, 533-536.
Slot, J. W. &H. J. Geuze 1985. A new method of preparing gold probes for multiple-labeling cytochemistry. Europ. J. Cell Biol. 38, 87-93.

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how to examine biological specimens with microscopes. Chapters include LM &TEM theory, colloidal gold production, immunocytochemistry, cryosectioning and more.

Cell Biology home page

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