

COLLOIDAL GOLD



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PRODUCT INFORMATION

COLLOIDAL GOLD-ANTIBODY AND PROTEIN CONJUGATES

Product Name: COLLOIDAL GOLD CONJUGATES
Catalog Number: CG0300...CG3054
Appearance: Brown (3 nm gold conjugates), red (5, 10, 15 nm gold conjugates) or purple (30 nm gold conjugates) solutions
Revision: 1.3 (March 2000)

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Warning: For research use only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals. Non radioactive and non carcinogenic.

PRODUCT INFORMATION

The Nanoprobes line of colloidal gold conjugates^{1,2} consists of affinity purified IgG antibodies or streptavidin adsorbed to colloidal gold particles with 3, 5, 10, 15, and 30 nm diameters. These conjugates are purified by size exclusion chromatography for minimal aggregates.

The optical density at 520 nm = 3.0 for 3,5, and 10 nm conjugates; 4.0 for 15 nm conjugates; and 5.0 for 30 nm conjugates. Except for the 30 nm conjugate, these conjugates come as solutions in 20mM Tris buffered saline, pH 8.2, with 1% bovine serum albumin and 0.05% sodium azide as stabilizers. The 30 nm conjugates are packaged with 0.1 % bovine serum albumin and 0.05% Carbowax as stabilizers.

Colloidal gold particle concentration and number of adsorbed antibodies

From spectroscopic measurements on freshly prepared colloidal gold sols of different sizes, we have calculated the concentrations of gold particles in our commercial preparations of colloidal gold. These values assume that all the tetrachloroaurate used for the preparation is converted to colloidal gold, and that the gold particles are composed of metallic gold of density 19.31 grams per mL:

Particle Size nm	Particle Volume (nm ³)	OD Value	Particles/mL	[Particles] nmol/mL
3	14.1	10.0 (at 360 nm)	-	-
5	65.5	3.0 (at 520 nm)	1.95 X 10 ¹⁴	0.32
10	523	3.0 (at 520 nm)	2.09 X 10 ¹³	0.034
15	1,767	4.0 (at 520 nm)	8.20 X 10 ¹²	0.013
30	14,140	5.0 (at 520 nm)	1.13 X 10 ¹²	0.0019

We have also calculated the approximate numbers of IgG molecules and streptavidin which can adsorb to our gold particles. These values are based on a contact area for IgG of 45 nm², and for streptavidin of 25 nm², and assume that the IgG or streptavidin molecules cover 50 % of the surface area of the gold particles:

Particle Size nm	Surface Area (nm ²)	IgG molecules per particle	[IgG] nmol/mL	Streptavidins per particle	[Streptavidin] nmol/mL
3	28.3	1	-	-	-
5	78.5	1.7	0.56	3.1	1.0
10	314	7.0	0.24	12.6	0.44
15	706	15.7	0.21	28.3	0.38
30	2,827	62.8	0.12	113.1	0.21

Please Note: these values are estimates based on assumptions, and have not been confirmed by experimental measurements of size, particle counting, or determination of adsorbed antibodies or streptavidin.

GENERAL CONSIDERATIONS FOR IMMUNOSTAINING WITH NANOPROBES COLLOIDAL GOLD CONJUGATES

Normal methodologies may be used successfully with colloidal gold immunoreagents. The concentration of antibody and gold is comparable to other commercial preparations of colloidal gold antibodies. Therefore similar dilutions and blocking agents are appropriate. Over staining with OsO₄, uranyl acetate or lead citrate may be conducted without obscuring the gold particles; for even higher contrast with cells or organelles in suspension, we recommend our NANOVAN³ and NANO-W negative stains.

ELECTRON MICROSCOPY IMMUNOLABELING WITH COLLOIDAL GOLD CONJUGATES

If aldehyde-containing reagents have been used for fixation, these must be quenched before labeling. This may be achieved by incubating the specimens for 5 minutes in 50 mM glycine solution in PBS (pH 7.4). Ammonium chloride (50 mM) or sodium borohydride (0.5 - 1 mg/ml) in PBS may be used instead of glycine.

Cells in Suspension

- Optional fixing of cells: e.g., with glutaraldehyde (0.05 - 1% for 15 minutes) in PBS. Do not use Tris buffer since this contains an amine. After fixation, centrifuge cells (e.g. 1 ml at 10⁷ cells/ml) at 300 X g, 5 minutes; discard supernatant; resuspend in 1 ml buffer. Repeat this washing (centrifugation and resuspension) 2 times.
- Incubate cells with 0.02 M glycine in PBS (5 mins). Centrifuge, then resuspend cells in PBS-BSA
- buffer (specified below) for 5 minutes.
- Place 50 - 200 µl of cells into Eppendorf tube and add 5 - 10 ml of primary antibody (or antiserum).
- Incubate 30 minutes with occasional shaking (do not create bubbles which will denature proteins).
- Wash cells using PBS-BSA as described in step 1 (2 X 5 mins). Resuspend in 1 ml Buffer 1.
- Dilute colloidal gold conjugate ~ 50 times in PBS-BSA buffer and add 30 ml to cells; incubate for minutes with occasional shaking.
- Wash cells in PBS-BSA as described in step 1 (2 X 5 mins).

- Fix cells and antibodies using a final concentration of 1% glutaraldehyde in PBS buffer for 15 minutes. Then remove fixative by washing with PBS buffer (3 X 5 mins).

PBS-BSA Buffer:

20 mM phosphate
 150 mM NaCl
 pH 7.4
 0.5% BSA
 0.1% gelatin (high purity)
Optional, may reduce background:
 0.5 M NaCl
 0.05% Tween 20

PBS Buffer:

20 mM phosphate
 150 mM NaCl
 pH 7.4

Negative staining may be used for electron microscopy of small structures or single molecules which are not embedded. Negative stain must be applied after the silver enhancement. NANOVAN³ and NANO-W negative stains are recommended for use with colloidal gold reagents for highest contrast imaging.

Thin Sections

Labeling with Colloidal Gold may be performed before or after embedding.⁴ Labeling before embedding and sectioning (the pre-embedding method) is used for the study of surface antigens, particularly small organisms such as viruses budding from host cells. It gives good preservation of cellular structure, and subsequent staining usually produces high contrast for study of the cellular details. Labeling after embedding and sectioning (the post-embedding method) allows the antibody access to the interior of the cells, and is used to label both exterior and interior features. The procedures for both methods are described below.

Thin sections mounted on grids are floated on drops of solutions on parafilm or in well plates. Hydrophobic resins usually require pre-etching. All commonly used embedding media may be used in Colloidal gold labeling experiments.

PROCEDURE FOR PRE-EMBEDDING METHOD:⁴

- Float on a drop of water for 5 - 10 minutes.
- Incubate cells with 1 % bovine serum albumin in PBS buffer at pH 7.4 for 5 minutes; this blocks any non-specific protein binding sites and minimizes non-specific antibody binding.
- Incubate with primary antibody, diluted at usual working concentration in PBS-BSA (30 mins - 1 hour, or usual time. Buffer formulations are given below.
- Rinse with PBS-BSA (3 X 1 min).
- Incubate with colloidal gold reagent diluted 1/40 - 1/200 in PBS-BSA with 1 % normal serum from the same species as the colloidal gold reagent, for 10 minutes to 1 hour at room temperature.
- Rinse with PBS-BSA (3 X 1 min), then PBS (3 X 1 min).
- Postfix with 1 % glutaraldehyde in PBS (10 mins).
- Rinse in deionized water (2 X 5 min).
- Dehydrate and embed according to usual procedure.
- Stain (uranyl acetate, lead citrate or other positive staining reagent) as usual before examination.

Silver enhancement may be performed before or after embedding (see below); it should be completed before postfixing or staining with osmium tetroxide, uranyl acetate or similar reagents is carried out.

PROCEDURE FOR POST-EMBEDDING METHOD:⁴

- Prepare sections on plastic or carbon-coated nickel grid. Float on a drop of water for 5 - 10 minutes.
- Incubate with 1 % solution of bovine serum albumin in PBS buffer at pH 7.4 for 5 minutes to block non-specific protein binding sites.

3. Incubate with primary antibody, diluted at usual working concentration in PBS-BSA (1 hour or usual time. Buffer formulations are given below).
4. Rinse with PBS-BSA (3 X 1 min).
5. Incubate with Colloidal Gold reagent diluted 1/40 - 1/200 in PBS-BSA with 1 % normal serum from the same species as the colloidal gold reagent, for 10 minutes to 1 hour at room temperature.
6. Rinse with PBS (3 X 1 min).
7. Postfix with 1 % glutaraldehyde in PBS buffer at room temperature (3 mins).
8. Rinse in deionized water for (2 X 5 min).

If desired, contrast sections with uranyl acetate and/or lead citrate before examination.

Silver enhancement may also be used to enlarge the colloidal gold particles (see below). Silver enhancement should be completed before stains such as uranyl acetate, osmium tetroxide or lead citrate are applied.

PBS-BSA Buffer:

20 mM phosphate
150 mM NaCl
pH 7.4
0.5% BSA
0.1% gelatin (high purity)
Optional, may reduce background:
0.5 M NaCl
0.05% Tween 20

PBS Buffer:

20 mM phosphate
150 mM NaCl
pH 7.4

SILVER ENHANCEMENT OF COLLOIDAL GOLD FOR EM

Colloidal gold will nucleate silver deposition resulting in a dense particle 2-80 nm in size or larger depending on development time. If specimens are to be embedded, silver enhancement is usually performed after embedding, although it may be done first. It must be completed before any staining reagents such as osmium tetroxide, lead citrate or uranyl acetate are applied, since these will nucleate silver deposition in the same manner as gold and produce non-specific staining.

Our LI SILVER silver enhancement system is convenient and not light sensitive, and suitable for all applications. Improved results in the EM may be obtained using HQ SILVER, which is formulated to give more controllable particle growth and uniform particle size distribution, at neutral pH for best possible ultrastructural preservation.⁵

Specimens must be thoroughly rinsed with deionized water before silver enhancement reagents are applied. This is because the buffers used for antibody incubations and washes contain chloride ions and other anions which form insoluble precipitates with silver. These are often light-sensitive and will give non-specific staining. To prepare the developer, mix equal amounts of the enhancer and initiator immediately before use. Colloidal Gold will nucleate silver deposition resulting in a dense particle 2-20 nm in size or larger depending on development time. Use nickel grids (not copper).

The relevant procedure for immunolabeling should be followed up to step 7 as described above. Silver enhancement is then performed as follows:

1. Rinse with deionized water (2 X 5 mins).
2. Float grid with specimen on freshly mixed developer for 1-8 minutes, or as directed in the instructions for the silver reagent. More or less time can be used to control particle size. A series of different development times should be tried, to find the optimum time for your experiment. With HQ Silver, a development time of 4 min. gives 15-40 nm round particles.
3. Rinse with deionized water (3 X 1 min).
4. Mount and stain as usual.

Fixing with osmium tetroxide may cause some loss of silver; if this is found to be a problem, slightly longer development times may be appropriate.

NOTE: Treatment with osmium tetroxide followed by uranyl acetate staining can lead to much more drastic loss of the silver enhanced NANOGOLD™ particles. This may be prevented by gold toning:⁶

1. After silver enhancement, wash thoroughly with deionized water.
2. 0.05 % gold chloride: 10 minutes at 4°C.
3. Wash with deionized water.
4. 0.5 % oxalic acid: 2 mins at room temperature.
5. 1 % sodium thiosulfate (freshly made) for 1 hour.
6. Wash thoroughly with deionized water and embed according to usual procedure.

IMMUNOLABELING AND SILVER ENHANCEMENT WITH COLLOIDAL GOLD FOR LIGHT MICROSCOPY

Features labeled with colloidal gold will be stained black in the light microscope upon silver enhancement. Different development times should be tried to determine which is best for your experiment. The procedure for immunolabeling is similar to that for EM; a suitable procedure is given below.

Samples must be rinsed with deionized water before silver enhancement. This is because the reagent contains silver ions in solution, which react to form a precipitate with chloride, phosphate and other anions which are components of buffer solutions. The procedure for immunolabeling with colloidal gold and silver enhancement is given below.

1. Spin cells onto slides using Cytospin, or use paraffin section.
2. Incubate with 1 % solution of bovine serum albumin in PBS (PBS-BSA) for 10 minutes to block non-specific protein binding sites.
3. Incubate with primary antibody, diluted at usual working concentration in PBS-BSA (1 hour or usual time)
4. Rinse with PBS-BSA (3 X 2 min).
5. Incubate with Colloidal Gold reagent diluted 1/40 - 1/200 in PBS-BSA with 1 % normal serum from the same species as the Colloidal Gold reagent, for 1 hour at room temperature.
6. Rinse with PBS (3 X 5 min).
7. Postfix with 1 % glutaraldehyde in PBS at room temperature (3 mins).
8. Rinse with deionized water (3 X 1 min).
9. Develop specimen with freshly mixed developer for 5-20 minutes, or as directed in the instructions for the silver reagent. More or less time can be used to control intensity of signal. A series of different development times may be used, to find the optimum enhancement for your experiment; generally a shorter antibody incubation time will require a longer silver development time.
10. Rinse with deionized water (2 X 5 mins).

The specimen may now be stained if desired before examination, with usual reagents.

PBS-BSA Buffer:

20 mM phosphate
150 mM NaCl
pH 7.4
0.5% BSA
0.1% gelatin (high purity)

Optional, may reduce background:

0.5 M NaCl
0.05% Tween 20

PBS Buffer:

20 mM phosphate
150 mM NaCl
pH 7.4

To obtain an especially dark silver signal, the silver enhancement may be repeated with a freshly mixed portion of developer.

IMMUNOBLOTTING

The basic procedure for gold immunoblotting has been described by Moeremans et al,⁷ which may be followed. For best results, the membrane should be hydrated before use by simmering in gently boiling water for 15 minutes. Best results are obtained when the antigen is applied using a 1 ml capillary tube. The procedure for immunoblots is as follows:

1. Spot 1 ml dilutions of the antigen in buffer 4 onto hydrated nitrocellulose membrane. Use an antigen concentration range from 100 to 0.01 pg / ml.
2. Block with buffer 1 for 30 minutes at 37°C.
3. Incubate with primary antibody according to usual procedure (usually 1 or 2 hours).
4. Rinse with buffer 1 (3 X 10 mins).
5. Incubate with a 1/100 to 1/200 dilution of the colloidal gold reagent in buffer 2 for 2 hours at room temperature.
6. Rinse with buffer 3 (3 X 5 mins), then buffer 4 (2 X 5 mins).
7. OPTIONAL (may improve sensitivity): Postfix with glutaraldehyde, 1 % in buffer 4 (10 mins).
8. Rinse with deionized water (2 X 5 mins).
9. OPTIONAL (may reduce background): Rinse with 0.05 M EDTA at pH 4.5 (5 mins).
10. Develop with freshly mixed silver developer for 20-25 minutes or as directed in the instructions for the silver reagent, twice. Rinse thoroughly with deionized water between developments to remove all the reagent.
11. Rinse several times with deionized water.

Buffer 1: 20 mM phosphate
150 mM NaCl
pH 7.4
4% BSA (bovine serum albumin)
2 mM sodium azide (NaN₃)

Buffer 3: 20 mM phosphate
150 mM NaCl
pH 7.4
0.8% BSA (bovine serum albumin)
2 mM sodium azide (NaN₃)

Buffer 2: 20 mM phosphate
150 mM NaCl
pH 7.4
0.8% BSA
1% normal serum; use serum of the host animal
for the colloidal gold-labeled antibody
0.1% gelatin (Type B, approx. 60 bloom)
Optional, may reduce background:
0.5 M NaCl
0.05% Tween 20

Buffer 4 (PBS):
20 mM phosphate
150 mM NaCl
pH 7.4

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Technical Assistance Available.

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