AURION GOLD SOLS

PRODUCT INFORMATION

AURION GOLD SOLS are based on the strictly defined particle sizes of 6, 10, 15 or 25nm. The sols are monodisperse, with a coëfficient of variance <15%, but in most cases <10%.
The actual lot specifications (size, variation and expiry date) are indicated on a separate Quality Control Sheet.
AURION GOLD SOLS have a guaranteed shelf life of one year after the date of quality control analysis.

The products should be stored at 4-8°C.
Do not freeze!

GENERAL REMARKS

Conjugation procedures can e.g. be found in the CRC Press-edition "Immunogold Labeling in Cell Biology", A.J. Verkley & J.L.M. Leunissen eds., (1989), Boca Raton, Florida.
In addition the issues of the Academic Press edition "Colloidal Gold", M.A. Hayat ed., (1989), San Diego, California are highly recommended.

INTRODUCTION TO GOLD CONJUGATE PREPARATION USING AURION GOLD SOLS

The most crucial factor for success is the determination of the iso electric point (pI) of the protein to be coupled . This value or an approximately 0.5 pH unit higher value is considered as the optimum pH for gold conjugate preparation. Under these conditions the net protein charge is zero or is slightly negative. The lowest amount of protein added to the gold sol preventing a colour change upon addition of an electrolyte solution is considered as the stabilizing amount. Remaining uncoated particle surface areas can be covered by adding secondary stabilizers. The surplus of free protein molecules has to be removed after gold conjugate preparation by ultracentrifugation.

DETERMINATION OF THE MINIMAL PROTECTING AMOUNT OF PROTEIN

The protein solution is dialysed against a very dilute (approximately 5 mM) salt solution or buffer at the appropriate pH, which corresponds to the pI value or about 0.5 pH unit higher. If possible, the solution is dialysed against distilled water.

Immunoglobulins are preferably dialysed against 2 mM Borax buffer or 5 mM $NaHCO_3$ at pH 9.0. The dialysed solution is centrifuged at 100,000xg for 1 hr and the protein concentration is determined. The protein concentration should be kept below 1 mg/ml to prevent the formation of di- and oligomers.

The pH of an appropriate amount of colloidal gold is adjusted likewise with either 0.2 N K_2CO_3 or 0.2 M H_3PO_4. The pH-electrode should be kept in contact with the sol for the shortest possible time, as the colloidal particles tend to plug the electrode diaphragm. Glass electrodes may be cleaned with aqua regia. Since gel-filled electrodes have a low electrolyte flow, these are preferred to standard electrodes.

Part of the protein stock solution is diluted in dialysis buffer corresponding to 0.5 mg/ml. Make 10 linear dilutions in 5ml plastic tubes according to Table 1:

Table 1

tube				
	1	100 µl protein solution	+	0 µl dialysis buffer
	2	90	+	10
	3	80	+	20
	4	70	+	30
	5	60	+	40
	6	50	+	50
	7	40	+	60
	8	30	+	70
	9	20	+	80
	10	10	+	90

Add 1 ml pH-adjusted gold sol to each tube. Vortex and allow to stand for 2 minutes. Next add 100 µl of a 10 % NaCl solution, vortex and allow to stand for 5 minutes. Measure the OD_{580nm} using as a blank: 1 ml gold sol with 100 µl dialysis buffer and 100 µl distilled water. The measured values are plotted to obtain a concentration variable adsorption isotherm (CVAI). The point at which the curve appears asymptotic with the abscissa denotes the minimal amount of protecting protein.

PREPARATION OF THE GOLD CONJUGATE AND FURTHER PURIFICATION

The required gold conjugate is prepared using the undiluted protein stock solution. After addition of the minimal amount of stock protein solution to the gold sol the mixture is allowed to stand for 2 minutes. In order to block remaining free surface areas on the gold particles and to prevent aggregation of the gold conjugate it is necessary to add a secondary stabilizer. To this end bovine serum albumin (adjusted to the same pH as used for conjuagtion) can be used. Add such an amount of a 10% BSA solution that the final concentration is 1%. In order to remove non-adsorbed protein, incompletely stabilized particles and aggregates the gold conjugate must be purified. This is generally achieved by centrifugation. Spin the gold conjugate down according to the data in Table 2 preferably at 4°C.

Table 2:

Particle diameter	gravity	time
6nm	$45,000xg_{av}$	45 minutes
10 nm	$45,000xg_{av}$	30 minutes
15 nm	$12,000xg_{av}$	45 minutes
25 nm	$12,000xg_{av}$	30 minutes

The pellets are partly solid and partly fluffy. The fluffy parts are collected and used for further purification. The solid pellets are not recovered as they are largely composed of aggregates. The non-adsorbed proteins remain in the supernatant. For light microscopy and blotting applications the fluffy pellets are resuspended in PBS with 1% BSA and 20 mM NaN_3, pH 7.6. The pelletting procedure may be repeated# and the fluffy pellets are once more collected. The gold conjugate is stored prior to use at 4°C at an OD_{520nm} of 1.0 - 2.0.

#When preparing immunoglobulin gold conjugates however it is advised to limit the pelleting to one centrifugation step. The reason for this is that clusters of immuno globulin gold conjugates are very easily formed by a repeated pelletting procedure. For electron microscopical applications a further purification and an increase in particle size homogeneity may be obtained by density centrifugation of the first fluffy pellets on a linear 10-30% glycerol or sucrose gradient in

buffer. After centrifugation the supernatant on the gradient is removed and the upper third of the gradient is collected. If necessary the glycerol is removed by dialysis against PBS with 1% BSA and 20 mM NaN_3. Further advantages of this procedure are the removal of aggregates of gold conjugates, the removal of non-adsorbed macromolecules left in the supernatant on top of the gradient and a high-yield preparation.
Table 3 summarizes gradient centrifugation data depending upon the particle diameter.

Table 3:

Particle diameter	gravity	time
6 nm	125,000xg_{av}	45 minutes
10 nm	50,000xg_{av}	45 minutes
15 nm	15,000xg_{av}	45 minutes
25 nm	10,000xg_{av}	45 minutes

After purification the gold conjugate OD_{520nm} is determined and adjusted as described. The gold conjugate is stored at 4°C prior to use. Addition of glycerol or sucrose to a final concentration of at least 20% or 2M respectively allows freezing of the gold conjugates in liquid nitrogen and storage at -80 or -20°C (Slot and Geuze, *J. Cell Biol.* , 90, (1981), 533)and even successful lyophilization has been reported (Baschong and Roth, *Histochem. J.*, 17, (1985), 1147).

EVALUATION OF GOLD CONJUGATE

The quality of a gold conjugate can be assessed from electron micrographs and from spot tests.

Electron microscopy can be used to evaluate the particle size, the size distribution and the presence of clusters on a quantitative basis. Gold conjugate can be attached to poly-L-lysine coated formvar or parlodion coated grids. The particle density per unit surface area should be kept low in order to be able to evaluate the presence of clusters. As a rule, gold conjugates with at least 75% singlets and at the most 5% triplets are considered to be acceptable.

The bioactivity of a gold conjugate can be assessed by examination of their applicability in immuno overlay techniques (Moeremans et al., *J. Immunol. Methods*, 74, (1984), 353). In such a test, a 1:10 - 1:20 dilution of the concentrated gold conjugate is reacted at room temperature until saturation with a nitrocellulose strip onto which spots containing a dilution series of the corresponding antigen (250-0.1 ng per spot) have been applied.

Visual evaluation of the red colour developed after the reaction gives quantitative data on the bioactivity of the gold conjugate.
Using this approach, the stability of the gold conjugate with time can also be determined. The working titre can be monitored by reacting a dilution series of the gold conjugate with spots of a fixed amount of antigen.

Although gold conjugates are in general quite stable, protein desorption from the gold particle surface may occur with prolonged storage. It is advised to check gold conjugate performance after longer periods of storage. In case of diminished performance a repeated purification may be necessary for instance by (gradient) centrifugation as described. Apart from possible desorption, the specific protein activity may and will deteriorate in time and should therefore be checked.

PRODUCT INFORMATION

AURION ST-US is based on ultra small atomic gold clusters (number of atoms/cluster < 30, average diameter < 0.8 nm) which have been stabilized with Bovine Serum Albumin fragments which may be covalently bound to (macro)molecules by e.g. glutaraldehyde cross-linking.
The ultra small cluster size (and associated reagent size) warrants optimum sensitivity and facilitated penetration.
AURION ST-US have a guaranteed shelf life of one year after the date of quality control analysis.

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Do not freeze!

AURION ST-US particles can be coupled to macromolecules by co-valent binding. A procedure based on glutaraldehyde coupling is described. During the procedure the mixtures will change in colour due to Schiff base formation.

1 Adjust the pH of an appropriate amount of AURION ST-US to 8.0 with 1M NaOH.
Likewise adjust the pH of a 10% glutaraldehyde solution (purest grade) to 8.0 with 1M NaOH.

2 Mix equal volumes of the pH adjusted AURION ST-US and the adjusted glutaraldehyde solution and stirr for 2 hours at 35 - 40°C in a hood.

3 Dialyse the mixture against 5mM $NaHCO_3$ overnight at 4°C with at least 4 changes.

4 Dissolve the specific protein to be bound in 5mM $NaHCO_3$ at a concentration of 0.2mg/ml.

5 Mix equal volumes of the specific protein solution and the dialysed glutaraldehyde activated AURION ST-US and stirr overnight at 4°C.

6 Add 1 ml of a 2M glycine solution, adjusted to pH 8.0 with NaOH. Stirr overnight at 4°C. This treatment results in inactivation of remaining free aldehyde groups.

The resulting conjugates may be purified by gel filtration chromatography.
The complexes may be further purified by affinity purification to separate specific protein/AURION ST-US conjugates from unbound AURION ST-US.

AURION
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