AURION DIAGNOSTISC GOLD SOLS

PRODUCT INFORMATION

AURION DIAGNOSTICS GOLD SOLS are based on the strictly defined particle sizes of 20 and 40nm. The gold nanoparticles are monodisperse, with a coëfficient of variance <12%. AURION DIAGNOSTICS GOLD SOLS are supplied at OD =1 (20nm OD_{520nm} =1, 40nm OD530nm =1). They are optimised for passive adsorption to high molecular weight molecules, e.g., antibodies. AURION DIAGNOSTICS 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

Procedures for conjugation via adsorption 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.

Procedures for gold nanoparticle functionalisation and covalent conjugation procedures are described in e.g., Bioconjugate Techniques, 3rd edition, G.T. Hermanson ed., (2013), Academic Press.

For information on gold nanoparticle conjugation strategies see our Newsletter #6

GOLD NANOPARTICLE CONJUGATION VIA ADSORPTION

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 (ultra)centrifugation or by ultrafiltration.

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 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 0.1 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 conjugation) in general can be used. Add such an amount of a 10% BSA solution that the final concentration is 1%. For 40nm gold conjugates an initial stabilising step, prior to the addition of BSA, with high MW polyethylene glycol (e.g., PEG 20kDa) in a final concentration of 0.05% may be advisable. In order to remove non-adsorbed protein, from 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
20nm	$12,000xg_{av}$	30 minutes
40 nm	$12,000xg_{av}$	10 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. The fluffy pellets are resuspended in PBS with 1% BSA and 20 mM NaN_3, pH 7.6. The gold conjugate is stored prior to use at 4°C at an OD_{520nm} of 1.0 - 2.0.
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 e.g., 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.

GOLD NANOPARTICLE FUNCTIONALISATION AND COVALENT CONJUGATION

AURION DIAGNOSTICS GOLD NANOPARTICLES have been developed for conjugation via the direct adsorption method. This conjugation method is especially suited for large MW molecules (MW > 40kDa). For smaller molecules, e.g., ligands, initial functionalization of the gold nanoparticle surface with thiolated polyethylene (PEG) polymers should be performed. The functional groups can be, a.o., carboxyl, maleimide and azido groups. With a strength of 47 kcal/mol (197 kJ/mol) the sulphur (thiol)-gold bond is considered to ensure prolonged stability of ligand-stabilized gold nanoparticles. However Hostetler et al. already described in 1999 that thiol bound ligands appear to be mobile, i.e., diffuse to some extend on/from the surface of metal particles. Prolonged storage of functionalized gold nanoparticles (prior to covalent conjugation) is therefore not recommended. One solution to increase the shelf life is freeze drying. To avoid particle aggregation large thiolated PEG polymers having a MW of 5kDa are being used.

Procedures for gold nanoparticle functionalisation and covalent conjugation procedures are described in e.g., Bioconjugate Techniques, 3rd edition, G.T. Hermanson ed., (2013), Academic Press.

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