Gold Conjugation Kit (Covalent Conjugation) Protocol

Kit Components

NHS-Activated Gold Nanoparticles (lyophilized)
Protein Re-suspension Buffer
Reaction Buffer
Quencher Solution

Factors to Consider before Conjugation

The protein/antibody or other ligand to be conjugated needs to be in a purified form, and proper care must be taken to ensure that the ligand stock is devoid of the following for proper functionality:

1. No additional protein additives such as BSA
2. Avoid free amino acids (e.g. glycine)
3. Avoid common thiol additives such as DTT, TCEP and mercaptoethanol
4. Avoid EDTA
5. Avoid primary amine containing buffers or components (e.g. Tris)
6. Avoid use of strong buffers that might change the pH of the conjugation reaction.

If your protein/antibody stock contains any of the above, dialyse or use a desalting column to transfer your ligand into a compatible buffer such as sodium phosphate, MOPS, MES or HEPES. If contaminating proteins such as BSA is present, the protein needs to be purified prior to conjugation.

Conjugation Procedure

A recommended starting protocol for conjugation can be found below. Note that the amount of protein added may need to be optimized for your particular protein.

1. Allow all reagents to warm to room temperature before use.
2. Using the supplied protein re-suspension buffer, dilute or dissolve your protein/antibody to the final concentration suitable for the particular gold nanoparticle size to be conjugated.

Note: For effective conjugation, the purity of the protein needs to be considered. Any other molecules containing primary amines (e.g. TRIS) or other contaminating proteins (e.g. BSA) may compete with the protein to be conjugated and hence severely reduce the conjugation efficiency and should therefore be avoided.

45-16 Ramsey Road Shirley, NY 11967, USA
Tel: 631-624-4882 ·Fax:631-614-7828
E-mail: info@creative-diagnostics.com
www.cd-bioparticles.com

3. In a microcentrifuge tube combine your diluted protein sample with reaction buffer according to the table below.

	3 or 10 Small Kits	Medium Kits
Reaction Buffer	60 µL	600 µL
Diluted Protein Solution	48 µL	480 µL
Total Volume	108 µL	1080 µL

4. Transfer 90 µL (900 µL for the Medium Kit) of your protein/reaction buffer mix prepared in step 3 to one of the vials containing lyophilized NHS-activated gold nanoparticles and immediately mix well by pipetting up and down.

Note: Do not resuspend the lyophilized NHS-activated gold nanoparticles in buffer prior to addition of protein. NHS rapidly hydrolyzes in aqueous solution and may result in loss of conjugation efficiency.

5. Incubate the vial at room temperature for at least 2 hours.
6. Add 10 µL (100 µL for Medium Kit) of quencher solution to the vial to stop the reaction.
7. Using a microcentrifuge, centrifuge the vial for 30 minutes using the appropriate speed for the gold nanoparticle size you are using according to the table below.

Size	Centrifugation Force	Time
5 nm	100 kDa MWC Spin Column	30 min
10 nm	17000 x g	60 min (~50% recovery)
15 nm	17000 x g	30 min
20 nm	6500 x g	30 min
30 nm	4500 x g	30 min
40 nm	2500 x g	30 min
50 nm	2000 x g	30 min
60 nm	1125 x g	30 min
80 nm	600 x g	30 min
100 nm	400 x g	30 min
150 nm	180 x g	30 min
200 nm	100 x g	30 min

8. Discard the supernatant containing unbound protein.
9. Add 100 uL (1 mL for Medium Kit) of gold conjugate storage buffer to the vial to re-suspend your conjugate.

Note: A gold conjugate storage buffer is not supplied with the kit. Use a standard biological buffer compatible with your protein.

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A recommended storage buffer for an antibody gold conjugate is 20 mM Tris (pH 8.0), 150 mM NaCl supplemented with 1% (w/v) BSA and 0.025% Tween 20.

10. Record the UV-VIS spectra of the conjugate using a spectrophotometer, and dilute to desired optical density using gold conjugate storage buffer.
11. Store your protein conjugate at 4°C until use.

Suggested Protein Concentration

Gold Nanoparticle Diameter	Suggested Protein Concentration
5 nm	5 mg/mL
10 nm	3 mg/mL
15 nm	2 mg/mL
20 nm	1 mg/mL
30 nm	1 mg/mL
40 nm	0.5 mg/mL
50 nm	0.5 mg/mL
60 nm	0.5 mg/mL
70 nm	0.5 mg/mL
80 nm	0.5 mg/mL
90 nm	0.5 mg/mL
100 nm	0.5 mg/mL

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