

Gold Conjugation Kit (Passive Adsorption) Protocol

Materials and Equipment Required

Standard Gold Nanoparticles
10% (w/v) NaCl
10% (w/v) PEG (5,000-20,000 Da)
Phosphate Buffered Saline, PBS
Protein to be conjugated
UV-VIS Spectrophotometer

Titration Procedure

This procedure determines the amount of protein needed to saturate and stabilize the gold colloid.

1. Transfer 250 μ L of resuspended gold nanoparticles and place in 1.5 mL Eppendorf tubes.
2. Adjust pH of the gold colloid to match the isoelectric point of the protein to be conjugated.
3. Add between 0 and 1 mg of protein in 25 μ L to the gold nanoparticles while mixing to titrate the amount needed to saturate the gold surface.
4. Incubate for 10-15 minutes at room temperature.
5. Add 250 μ L of 10% NaCl solution.
6. Observe color change and determine which concentration the gold nanoparticle surface is saturated and no aggregation occurs upon addition 10% NaCl. This can be observed by an increase in absorbance at 580 nm compared to the control.

Note: The amount of protein needed to saturate the gold colloid can also be determined by agarose gel electrophoresis due to the change in charge upon binding of the protein.

Conjugation Procedure

1. Transfer amount of gold nanoparticles needed for your application from the stock to a new tube.
2. Add protein amount as determined above plus an additional 10%.
3. Incubate for 30 minutes at room temperature while stirring.
4. Centrifuge the solution for 30 minutes at the appropriate speed for the gold nanoparticle size used.
5. Resuspend the pellet in PBS supplemented with 0.1% BSA or 1% PEG.
6. Store at 4°C until use.

Suggested Centrifugation Conditions

Appropriate G forces for centrifugation of gold nanoparticles. Listed conditions are for a volume of 1 mL and centrifugation using a microcentrifuge, except for 5 nm gold nanoparticles that requires an ultracentrifuge.

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