ADSORPTION OF PROTEINS TO GOLD NANOPARTICLES – TECH NOTE: #102

Background:

Gold nanoparticle conjugates have been used for a wide range of biological applications including the use as probes in light and electron microscopy. Stable gold nanoparticle conjugates can easily be prepared by passive adsorption due to electrostatic and hydrophobic interactions between the protein and the surface layer of the colloidal gold. This process is maximally achieved at a pH close to the pI of the protein to be conjugated. An important parameter to consider when preparing gold nanoparticle conjugates is the amount of protein bound to the gold colloid. If too little protein is adsorbed to the gold surface, aggregation occurs upon addition of electrolytes present in standard buffers. A titration is therefore performed to determine at which protein concentration saturation and colloidal stability is reached.

Materials and Equipment Required:

- Standard Gold nanoparticles, Cat. # G-XX-XX
- 10% NaCl
- 10% PEG (5,000-20,000Da) or 10% BSA
- PBS
- Protein of interest
- UV-VIS Spectrophotometer

Titration procedure to find amount of protein needed to saturate and stabilize the gold colloid:

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

Related Product

Gold Nanoparticle Conjugation Optimization Kits – Complete systems to optimize pH and protein concentrations for passive adsorption of proteins to our standard and reactant-free gold nanoparticles.

Cytodiagnostics also provides conjugation of proteins and oligonucleotides to gold nanoparticles as a custom service.

Preparation (scale-up) of the gold nanoparticle conjugate:

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. For more information on appropriate centrifugation speeds of gold nanoparticles of different sizes, see Tech Note 101 – Storage and Handling.
5. Resuspend the pellet in PBS supplemented with 0.1% BSA or 1% PEG.
6. Store at 4°C until use.
References

Journal of Immunological Methods 356, 60-69