Gold Coating Hints

Number of Molecules that can be coated on a 40 nm gold particle:

A 40 nm gold particle can be coated with approximately 210 molecules of IgG per gold sphere. The saturation point for 40 nm gold particles is about 40 microgram of antibody per mL of 20 OD gold (or, 20 µl of antibody at a concentration of 2 mg/mL added to 1.0 mL 40 nm gold). The smaller the gold (e.g. 20 nm versus 40 nm) , the fewer antibody molecules can be coated on the sphere. However, since the smaller gold contains many more gold molecules per mL, in the end, more antibody is required for coating. For example, 20 nm gold at 20 OD requires 80 micrograms of antibody for coating.

The calculation for the amount of antibody that can be coated on each gold sphere is as follows:

There are 3.75 E15 molecules of antibody per 1.0 mL when the antibody is at a concentration of 1 mg per mL.

Twenty-two (22) mL of 20 OD gold contains 3.52 E 13 molecules of gold; thus each sphere saturates at nearly 210 molecules of antibody.

Number of gold particles in 40 nm – 20 OD gold per mL.

In 1 mL of 40 nm - 20 OD gold particles, there are approximately 1.6 x 10^12 gold spheres. (The number of gold spheres is related to both the nm size and the optical density (OD).

Conjugate yield per mg of antibody for 20 OD gold :

One (1.0) mg of antibody will yield approximately twenty-two (22) mL of 20 OD gold (40nm). During development, two or three mL of the 22 ml is consumed during fine tuning experiments.

Key Points for labeling gold particles to ligands:

The key to binding antibody to gold sols is the ability to covalently attach antibodies to intensely colored, nanometer particles. Gold sols that bind ligands through a sulfur bond have proved highly successful for this application. For optimal binding of the antibody or protein while retaining a high degree of specific activity, the pH of the gold sol must be adjusted to slightly above the iso-electric point of the coating antibody or protein.

Background:

Nano-gold particles remain in solution because they repel each other due to a significant negative charge. This means that proteins bind to gold particles through both ion-exchange attraction and covalent bonding of protein thiols (-SH) with surface gold. The challenge for preparing stable gold conjugates depends upon one's ability to manage the binding of antibody or proteins at or near their iso-electric point. In a few cases, the titration of the pH may need to be fine tuned.

Proteins with no surface thiol groups:

Antibodies or proteins in the sample must display a suitable number of surface thiols (-SH). Proteins with no surface thiol groups bind exchangably with gold particles through ion-exchange interactions. Such proteins do not form stable gold sols that are suitable for flowing chromatographic assays. Equally problematic are protein preparations where surface thiols have been capped or protected by reaction with N-ethyl maleimide or iodoacetic acid.

Hydrophobic antibody labeling:

Antibodies with multiple hydrophobic amino acids such as lysine, cysteine, tryptophan, leucine, isoleucine, methionine, valine and phenylalanine can be somewhat difficult to coat, and can aggregate the gold particles. Usually, a good blocker is needed to stabilize gold particles once the gold has been coated with hydrophobic antibody.

Often, mouse IgG 2A and IgG3 are difficult to coat due to a high iso-electric point. Rabbit polyclonal antibody can be somewhat tricky, while goat polyclonal is quite routine.

A sensitive lateral flow assay requires that all of the antibody or protein that is added to the gold sol is irreversibly bound to the beads. Any free antibody or protein will short-circuit the assay.