Gold Colloids

Preparation of antibody / protein gold conjugates

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Materials

All glassware must be scrupulously clean. Glass and plastic containers and stirrers should be cleaned in aqua regia, thoroughly washed in deionised water, and siliconised.

All reagents must be of high quality Analar grade and should be filtered immediately before use. Water should be double distilled or high quality deionised.

Antibody

The antibody should be affinity purified and of the highest quality. On the day of preparation make up a 0.1µg/µl solution of antibody in 2mM borax and dialyse for at least 4 hours in 1 litre of borax at pH9. Centrifuge the antibody at 100,000g for 1 hour at 4°C just before use. Keep at 4°C.

Gold Colloid

Procedures for the preparation of gold colloids of various article sizes are published elsewhere¹. A wide range a high quality gold colloids ready to use for conjugation to proteins is available from BBIInternational. For the example given here it is assumed that the gold colloid is prepared from 0.01% gold chloride solution.

Buffers
TBS
(1% bovine serum albumin (BSA) should be added to the base buffer)
Aqua regia
Titration

Conjugation of antibodies to gold particles depends upon three separate but dependent phenomena: (a) ionic attraction between the negatively charged gold and the positively charged protein; (b) hydrophobic attraction between the antibody and the gold surface; (c) dative binding between the gold conducting electrons and sulphur atoms which may occur within amino acids of the protein.

In order to form a strong absorption between gold and antibody a preliminary titration must be performed to determine the optimum conditions for conjugation.

- Adjust the gold colloid to pH 9.2. Pipette 1ml of colloid into each of a series of 3ml clean plastic tubes.
- Adjust the antibody (0.1µg/µl) to pH 9.0 with 100mM K₂CO₃ or 100mM HCl.
- Add the antibody to each tube in a series from 0 – 150µl (i.e. 0 – 15µg in steps of 0, 1, 2, 3, to 15µg).
- Make up each tube to 1.15ml with 2mM borax.
- Shake each tube and leave for approximately 5 minutes to conjugate.
- To each tube add 100µl of 10% NaCl and agitate for 1 minute.

The tube containing the minimum amount of protein required to stabilise the gold sol is indicated by the one in which the colour of the gold sol does not change from red to blue upon the addition of NaCl.

Determination of pH for conjugation

In addition to the titration for judging the minimum amount of protein is also necessary to determine the correct pH for the conjugation. This is best performed at, or near to the isoelectric point of the protein. It is found by performing the preliminary titration at different pH values (e.g. pH 7, 8, 9, 10) and examining the conjugate (without added salt) in the electron microscope. A spread of the gold particles with absorbed antibody is made by floating a Formvar coated nickel EM grid on a droplet of the conjugate for 30 minutes and then washing in deionised water. The suspension on the grid is observed without further treatment in the EM.

The correct pH for conjugation is that in which the conjugation of protein to gold does not produce clusters in the EM.

Final Conjugation

Having determined the minimum amount of protein to stabilise the gold sol the amounts may be scaled up. Typical volumes are described here.

1. Take 100ml of gold sol and adjust to pH9 (see note).
2. Adjust the dialysed and centrifuged antibody solution (0.1µg/µl) to pH9 (see note).
3. Add the determined amount of antibody solution dropwise to the gold while stirring rapidly.
4. After 5 minutes add 10ml of filtered 10% BSA at pH 9 and stir gently for 10 minutes

**Purification**

The gold conjugate must be purified from excess antibody and any small clusters removed before concentrating and storing.

1. Spin the gold conjugated at the following speeds according to gold particle size:
   - 5nm 45,000g for 1 hour at 4°C
   - 10nm 25,000g for 1 hour at 4°C
   - 15nm 15,000g for 1 hour at 4°C
   - 20nm 8,000g for 1 hour at 4°C
   - 30nm 6,000g for 1 hour at 4°C
   - 40nm 4,000g for 1 hour at 4°C
   These speeds and times are approximate and must be determined experimentally.
2. The gold conjugate will form a loose precipitate at the bottom of the tube. Discard the clear supernatant and re-suspend the pellet into 2ml of TBS.
3. Prepare a 10 - 30% glycerol gradient (10ml) in buffer A and carefully load the suspended conjugate onto the top.
4. Spin the gradient in a swing out rotor as follows according to particle size:
   - 5nm 125,000g for 1 hour at 4°C
   - 10nm 50,000g for 1 hour at 4°C
   - 15nm 15,000g for 1 hour at 4°C
   - 20nm 12,000g for 1 hour at 4°C
   - 30nm 9,000g for 1 hour at 4°C
   - 40nm 6,000g for 1 hour at 4°C
   These speeds and times are approximate and must be determined experimentally.
5. Carefully pipette out each fraction in 2ml fractions from the tube and examine in the EM as above. The clustered fractions will be found in the lower fractions.
6. Test each fraction for sensitivity by immunoblotting against the specific antigen blotted onto nitro-cellulose or on serially cut tissue sections.
7. Pool those fractions that are acceptable and suspend in TBS to a
dilution having an optical density of 3.0 at A520. Alternatively dilute in TBS to make a calculated protein concentration of approximately 30µg/ml from the original volume.

**Storage**

The gold conjugate, if correctly made, will be stable at 4°C for several months. By adding 20% glycerol to the final buffer, it is possible to freeze at -20°C for long term storage.

**Technical support**

Gold conjugates made as described here may be used according to the directions given in the Conjugate Technical Instruction Section.

**Reference**


**Product Information**

Gold Colloids
Bovine Serum Albumin