Silver Conjugation Kit (Passive Adsorption) Protocol

Material and Equipment Required

Standard Silver Nanoparticles
10% NaCl (w/v)
2 mM sodium citrate tribasic dihydrate
10% Tween 20 (w/v)
Bovine Serum Albumin (BSA)
10X Phosphate Buffered Saline, 10X PBS
UV-VIS Spectrophotometer

Determination of pH and Protein Concentration

1. Aliquot 200 µL of silver nanoparticles into 1.5 mL tubes (200 µL for each condition to be tested).
2. Adjust the pH of the silver nanoparticle solution to the desired pH (optimal pH is generally close to the pI of the protein to be conjugated).
3. Add between 0 and 50 µg of protein in 10 µL to the silver nanoparticles and mix well to determine the amount needed to saturate the silver surface.
4. Incubate for 10 minutes at room temperature
5. Add 200 µL of a 10% NaCl stock solution and incubate for 10 minutes at room temperature.
6. Determine at which protein concentration the silver nanoparticle surface becomes saturated and no aggregation occurs upon addition of 10% NaCl by observing the color change and measuring the samples using a UV-VIS spectrophotometer. Degree of aggregation can be measured by an increase in absorbance at 690 nm and a decrease in absorbance at 405/480 nm (particle size dependant, see silver nanoparticle properties) compared to that of the non-conjugated control particles.

Note: The amount of protein needed to saturate the silver colloid can also be determined and verified through agarose gel-electroporesis. Binding of protein to the silver nanoparticle surface changes the overall particle charge and size both of which will affect the migration pattern in the agarose gel.

Conjugation Procedure

1. Transfer the desired volume of silver nanoparticles to 1.5 mL tubes.
2. Add Tween 20 to a final concentration of 0.025% (w/v).
3. Centrifuge the solution to pellet the silver nanoparticles.
4. Resuspend the silver nanoparticles with 2 mM sodium citrate to the original silver colloid volume and concentration.

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5. Adjust the pH of the silver nanoparticle solution as determined in the titration procedure above.
6. Add the appropriate amount of protein as determined in the titration procedure above plus an additional 10%.
7. Incubate for 60 minutes at room temperature on a rotary shaker/rocker.
8. Centrifuge the vial for 30 minutes at the appropriate speed for the silver nanoparticle size that you are conjugating to pellet the particles and remove the supernatant.
9. Resuspend the pellet in 1X PBS supplemented with 1% BSA (w/v).
10. Sonicate briefly in a sonicator bath to aid in dispersion if particles are partially agglomerated.
11. Validate the functionality of the final silver conjugate.
12. Store the silver conjugate at 4°C until use.

Suggested Centrifugation Conditions

Listed conditions are based on a 1 mL sample volume in 1.5 mL microcentrifuge tubes.

Size	Centrifugation Force	Time
10 nm	21000 x g	60 min
20 nm	17000 x g	30 min
30 nm	11000 x g	30 min
40 nm	3000 x g	30 min
50 nm	1500 x g	30 min
60 nm	900 x g	30 min
70 nm	700 x g	30 min
80 nm	500 x g	30 min
90 nm	400 x g	30 min
100 nm	300 x g	30 min

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