Passive Conjugation of Antibodies to i-colloid 40 nm Gold

(For Cat # AU40-1-50, AU40-1-100, AU40-5-50, AU40-5-100, AU40-5-200, and AU40-10-100)

Overview

This general protocol describes passive conjugation of antibodies to i-colloid 40 nm Gold nanoparticles provided at OD1, OD5, or OD10. The optimal pH and protein-to-gold ratio are antibody-dependent, and a conjugation optimization protocol is provided on the reverse side to determine these values prior to performing this protocol.

Reagents and Equipment

The antibody quantity and buffer formulations below are general recommendations, and different conditions may be required for optimal results. Refer to Notes.

- 5 mL i-colloid 40 nm Gold nanoparticles
- Antibody in low-salt buffer:
  30 µg for OD1, 150 µg for OD5, 300 µg for OD10
- Conjugation Buffer:
  100 mM phosphate buffer, pH 7.4
- Blocking Buffer:
  4 mM phosphate buffer, pH 7.4 + 10 mg/mL BSA
- Storage Buffer:
  4 mM phosphate buffer, pH 7.4 + 5 mg/mL BSA
- De-ionized (DI) water
- 15 mL centrifuge tubes
- Orbital shaker or rotisserie
- Vortex (optional)

Notes:

1. Conjugation optimization (protocol on reverse side) is recommended to determine appropriate antibody quantity and buffer formulation. Multiply the antibody concentration value determined during optimization by 50 (for OD1), 250 (for OD5), or 500 (OD10) to calculate the µg quantity needed for this procedure.

2. Conjugation typically performs well in buffer at a pH slightly above protein pI.

3. 100 mM Conjugation Buffer Preparation
   - For pH ≤ 8 dissolve sodium phosphate monobasic in DI water and titrate with 1M NaOH
   - For pH > 8 dissolve boric acid in DI water and titrate with 1M NaOH

4. Blocking Buffer Preparation
   Dilute Conjugation Buffer to 4 mM, add BSA to 10 mg/mL

5. Storage Buffer Preparation
   Dilute Conjugation Buffer to 4 mM, add BSA to 4 mg/mL

6. Purifying antibodies by de-salting may improve conjugation performance.

7. Perform parallel 15 mL reactions for larger quantities.

Procedure:

1. Rinse a 15 mL tube with DI water to remove trace impurities or contaminants.

2. Add 0.213 mL of Conjugation Buffer to the tube.

3. Add 5 mL of gold nanoparticles to the tube and mix by pipetting.

4. Rapidly add protein to the tube and immediately mix by vortex or pipet.

5. Cover tube and gently shake at 100 rpm or rotate for one hour at room temperature.

6. Add 5 mL Blocking Buffer to tube and gently shake/rotate for 30 minutes.

7. Centrifuge at room temperature for 30 minutes at 3000 xg.

   For conjugations with OD5 or OD10 gold nanoparticles, some conjugates may still be in solution after 30 minutes as indicated by a slightly colored supernatant. Additional centrifugation time can improve recovery.

8. Carefully remove supernatant and re-suspend conjugates with 5 mL of Storage Buffer.

9. Centrifuge at room temperature for 20 minutes at 3000 xg.

10. Carefully remove supernatant and re-suspend in 5 mL of Storage Buffer for conjugate concentration (by OD) equivalent to the starting gold concentration. Adjust Storage Buffer re-suspension volume appropriately if other conjugate concentrations are needed.

11. Transfer conjugate to fresh tube or vial pre-rinsed with DI water.

    Polypropylene tubes and silanized glass vials are recommended for conjugate stability.

12. Store conjugate at 4 °C until use. DO NOT FREEZE.

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Conjugation Optimization for Passive Adsorption of Antibodies to i-colloid Gold

Overview

The optimal concentration and pH for passive adsorption of antibodies to i-colloid gold nanoparticles is determined by assaying conjugate resistance to salt-induced aggregation. Conjugate particles with only partial surface antibody coverage will be sensitive to aggregation, while particles with full coverage will be resistant to aggregation. This protocol may also be applied to other proteins besides antibodies.

Reagents and Equipment

- i-colloid gold nanoparticles, OD 1
- Antibody (at least 1 mg/mL concentration)
- 1x PBS
- 100 mM buffers representing a range of 12 different pH between 5.7 and 9.8; 40 µL at each pH (See Notes regarding buffer formulations)
- 10% w/v NaCl, 10 mL

- Microfuge tubes
- Pipets (multi-channel pipets and reservoirs recommended for large number of optimization conditions)
- 96-well microplate
- Orbital shaker or rotisserie
- Absorbance plate reader (optional)

Notes:

1. This protocol can been performed in full 96-well plate format (8 antibody concentrations x 12 pH) and can be scaled to a lower number of concentrations and pH conditions.
2. Absorbance is recommended in addition to visual analysis for more quantitative evaluation.
3. Prepare OD1 gold from OD5 or OD10 by diluting with 50 µM NaCl.
4. 100 mM Buffer Preparation at various pH
   - For pH ≤ 8 dissolve sodium phosphate monobasic in de-ionized water and titrate with 1M NaOH
   - For pH > 8 dissolve boric acid in de-ionized water and titrate with 1M NaOH
5. Antibodies should be purified from sodium azide or BSA if present in antibody storage buffer.
6. This protocol may be applied to other proteins and may require testing a different range of concentrations.

Procedure:

Part I: Diluted Antibody Preparation

1. Select eight antibody concentrations to test a range of antibody-to-particle conjugation ratios, including a 0 µg/mL control. For IgGs, a 0-12 µg/mL range is recommended.
2. For each concentration, prepare 30 µL solution in 1x PBS at 50x the final reaction concentration (e.g. to test 6 µg/mL condition, prepare 30 µL of 300 µg/mL solution).
3. For each solution prepared above, dispense 2 µL per well to fill a designated row of a 96-well microplate (each row will correspond to a different antibody concentration).

Part II: Buffered Colloid Preparation

1. For each of the 100 mM buffers at various pH, prepare a tube of buffered colloid solution by mixing 40 µL buffer with 940 µL of OD 1 nanoparticles by pipetting.
2. For each buffered colloid solution, dispense 98 µL per well to fill a designated column of the microplate prepared in *Part I* and mix well by pipetting. Each well will now contain 100 µL of buffered colloid-antibody mixture at a unique pH and antibody concentration.

Part III: Buffered Colloid-Antibody Incubation and Analysis

1. Cover the microplate and shake at 100 rpm for one hour at room temperature.
2. Visually inspect the plate and confirm that all 0 µg/mL control wells appear pink/red. Wells that appear pink/red indicate no or little particle aggregation, while wells that appear lighter and or purple indicate particle aggregation.

Part IV: NaCl Testing and Analysis

1. Add 100 µL of 10% NaCl to each well.
2. Cover the microplate and shake at 100 rpm for one hour.
3. Visually inspect the plate and confirm that all 0 µg/mL control wells now appear lighter or have a purple tint.
4. If performing visual analysis only, select optimal antibody concentration and buffer pH by the following steps:
   a. Identify pH values that result in pink/red wells;
   b. Among pH values from (a), select pH slightly higher than antibody pI (if known) or one in middle of range;
   c. For pH chosen in (b), select lowest antibody concentration that results in pink/red wells.

Absorbance Analysis (best performed with plate reader)

1. Obtain absorbance measurements at 530 nm and 690 nm for each well (concentration + pH combination) and then calculate the corresponding A_{690}/A_{530} ratio ("aggregation index" or AI).
2. A large AI indicates conjugation reaction conditions that resulted in salt-induced particle aggregation due to incomplete antibody surface coverage.

   Generally:
   AI < 0.2 indicates little or no particle aggregation
   AI between 0.2 and 0.4 indicates some aggregation
   AI > 0.4 indicates severe aggregation
3. Select antibody concentration and buffer pH for large-scale conjugation by the following steps:
   a. Identify pH values that produce AI < 0.2;
   b. Among pH values from (a), select pH slightly higher than antibody pI (if known) or one in middle of range;
   c. For pH chosen in (b), select lowest antibody concentration that results in AI < 0.2.

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