Antibody Functionalization

Part Numbers:
C1x-Ab

Procedure

Generally, a 100 to 500-fold molar excess of targeted antigen over the molarity of antibody-bound gold nanoparticles is sufficient to drive the reaction. Vortexing is recommended to drive the reaction.

Antibody loading is listed on the COA per nanoparticle. The total concentration of the nanoparticles is also listed on the COA.

A. Material Preparation

Conjugation Buffer: PBS, Thermo Pierce 28372 non-potassium containing buffer between pH 6.5 and 7.5.

- Tabletop centrifuge.
- Target antigen.

B. Protocol

1. **Add antigen-containing target to antibody-terminated gold nanoparticles.** The product comes in densities of 2.5 mg/mL. We recommend using >1 mg/mL for the next steps.
2. **Sonicate to resuspend gold nanoparticles into solution.**
    - We typically use a Branson 5510 Ultrasonic Cleaner/Water bath or a Cole Parmer 08890-01 42kHz 1-2 Amps for 30 seconds.
3. **Vortex for 30 minutes at room temperature, up to 30°C for faster conjugations.**
4. **Purify by centrifugation.**
    - Centrifugation speeds depend on the centrifuge, but in general:
        - **Nanorods:** 8500 to 12000 rcf for 10 minutes
        - **Spheres:** 1500-15000 rcf
5. **Product rcf:**
    - 10 nm rods: 11000
    - 25 nm rods: 8500
    - Spheres 100 nm: 1500
    - Spheres 50 nm: 4000
    - Spheres 10 nm: 12000

6. **Repeat purification 3x.**
    - Refill with a 1% PBS 0.1% Tween solution. (1% PBS means standard PBS diluted 100x.)
    - In the final centrifugation, refill with 100% PBS.

Conjugation efficiency may be estimated by electrophoretic separation and subsequent protein staining.

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