

INSTRUCTIONS

Adsorbing Antibodies to Nanopartz Spherical Gold Nanoparticles for use in Lateral Flow

Part Numbers:

CL - Lateral Flow products

Introduction

Adsorbing antibodies to citrate-stabilized spherical gold nanoparticles (AuNPs) is a common procedure in nanobiotechnology, often used in the development of biosensors, diagnostics, and therapeutics. Below is a general protocol for adsorbing antibodies to citrate-stabilized spherical gold nanoparticles.

Important Product Information

In order to minimize these reversible occurrences, please make sure to store and handle your product as recommended in the Storage and Handling Instructions.

<u>Materials</u>

Citrate-stabilized spherical gold nanoparticles (AuNPs)

Antibodies (specific to your target)

Phosphate-buffered saline (PBS)

Bovine serum albumin (BSA) or other blocking agent

Centrifuge

Microcentrifuge tubes

pH meter or pH paper

Tween-20 (optional)

Sonicator: Branson 5510 Ultrasonic Cleaner/Water bath or a Cole Parmer 08890-01 42kHz 1-2 Amps

Mixer: Table top mixer, typically a pad with a touch activated rotation device.

Procedure

1. Preparation of Gold Nanoparticles:

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INSTRUCTIONS

- a. Ensure your gold nanoparticles are well-dispersed and free from aggregates. If necessary, sonicate the nanoparticle suspension for a few minutes to achieve a uniform dispersion.
- 2. Adjusting pH:
 - a. Adjust the pH of the AuNP solution to the optimal pH for antibody adsorption. This is typically around pH 7-8. You can use a dilute NaOH or HCl solution to adjust the pH.
- 3. Antibody Preparation:
 - a. Dilute the antibody to the desired concentration in PBS. Typical concentrations range from 0.1 mg/mL to 1 mg/mL.
- 4. Mixing Antibodies with AuNPs:
 - a. Slowly add the antibody solution to the AuNP solution while gently stirring. The typical volume ratio of AuNPs to antibody solution is 9:1.
 - b. For example, if you have 900 μL of AuNPs, add 100 μL of antibody solution.
 - c. Stir gently to ensure thorough mixing.
- 5. Incubation:
 - a. Allow the mixture to incubate at room temperature for 1-2 hours. This allows the antibodies to adsorb onto the surface of the gold nanoparticles.
- 6. Blocking (Optional but Recommended):
 - a. To prevent non-specific binding, add BSA or another blocking agent to the mixture. A common concentration is 1% BSA.
 - b. Incubate for an additional 30 minutes to 1 hour at room temperature.
- 7. Washing:
 - a. Centrifuge the mixture at the correct speed for 10-20 minutes to pellet the antibody-conjugated AuNPs.
 - b. Carefully remove the supernatant and resuspend the pellet in PBS. This step removes unbound antibodies and excess blocking agent.
 - c. Repeat the washing step 2-3 times to ensure thorough removal of unbound materials.



INSTRUCTIONS

8. Storage:

- a. After the final wash, resuspend the antibody-conjugated AuNPs in a small volume of PBS with 0.01% Tween-20 (optional, to prevent aggregation).
- b. Store the conjugated nanoparticles at 4°C. Avoid freezing.

Important Considerations:

- Antibody Concentration: Optimizing the concentration of antibodies is critical. Too high a concentration can lead to aggregation, while too low a concentration might not provide sufficient coverage.
- 2. Blocking Agents: BSA is commonly used, but other blocking agents can be used depending on the application and compatibility.
- 3. Stability: Monitor the stability of the antibody-conjugated AuNPs over time. Some formulations might require additional stabilizing agents.
- 4. Troubleshooting:
- 5. Aggregation: If aggregation occurs, try lowering the concentration of antibodies or adjusting the pH. Additionally, ensure that the washing steps are gentle to prevent physical disruption of the conjugates.
- 6. Low Yield: If the yield of functional antibody-conjugated AuNPs is low, consider optimizing the incubation time, antibody concentration, and washing conditions.

This protocol provides a general framework for adsorbing antibodies to citrate-stabilized gold nanoparticles. Depending on your specific application and the characteristics of the antibodies and nanoparticles, some optimization might be necessary.