Express Gold Conjugation Kit – Cat #: ACAU40-2, ACAU40-10

Overview

The Express Gold Conjugation Kit contains all reagents needed to stably conjugate antibodies to i-colloid® 40 nm gold nanoparticles coated with AnteoBind™ polymer (Activated Gold) without time-consuming pH optimization.

These instructions outline a general procedure to produce 50 µL of ~OD20 gold conjugates from 0.5 mL of OD2 Activated Gold using 2.5-12.5 µg of antibody.

Note: *Volumes and quantities may be scaled up as required. Longer centrifugal times may be required for larger volumes.*

Supplied Materials

Reagent	ACAU40-2 (mini)	ACAU40-10 (midi)
Activated Au (OD 2)	2 mL	10 mL
Wash Buffer	15 mL	30 mL
Conjugation Buffer	15 mL	30 mL
Storage Buffer	15 mL	30 mL

Required Materials & Equipment

- Antibody (prepared at ≥ 250 µg/mL in low-salt buffer for conjugation)
- BSA (recommended use at 10% w/v for blocking; *other blockers may be used*)
- Polypropylene microcentrifuge tubes (low protein binding)
- Pipettes
- Pipette tips (low protein binding - ThermoFisher Cat. No. 94052430 recommended)
- Microcentrifuge (capable of 2,000 x g)
- Rotary bench-top tube mixer (≈ 2-10 rpm)
- UV-Vis spectrometer
- Vortex mixer
- Bath ultrasonicator (optional)

Storage, Handling, and Safety Precautions

Store the kit refrigerated at 2–8 °C.

DO NOT FREEZE

Use standard precautions and PPE (gloves, lab coats) when handling laboratory reagents. Dispose of waste according to local, state and federal laws. Consult SDS for hazard information.

Procedure

Ensure reagents are equilibrated to room temperature before use. Always use a pipette to remove supernatants, taking care not to disturb the particle pellet.

BEFORE STARTING, READ NOTES ON REVERSE for steps to detect and reverse particle aggregation that may occur in an antibody- and quantity-dependent manner during incubation and centrifugation steps. Always observe solution color or pellet morphology at these steps and refer to superscript numbers in Notes if needed.

Antibody Conjugation to Activated Gold Nanoparticles

1. Fully disperse Activated Gold by vortex mixing gently for 10 seconds.[1] Transfer 0.5 mL to a microcentrifuge tube and centrifuge at 2,000 x g for 10 minutes.
2. Carefully remove 0.45 mL of the supernatant.[2] Explain how to remove w/o disturbing pellet
3. Add 0.95 mL of Wash Buffer and re-suspend the pellet by pipetting or gentle vortex mixing for 10 seconds.
4. Centrifuge the re-suspended particles at 2,000 x g for 10 minutes.
5. Carefully remove 0.95 mL of the supernatant.[2] Add 0.45 mL of Wash Buffer and re-suspend the pellet by pipetting or gentle vortex mixing for 10 seconds.
6. Prepare 0.5 mL of Antibody Solution at ***5 to 25 µg/mL*** in Conjugation Buffer in a fresh tube.[3]

   Note: *Optimize antibody concentration according to the assay requirement.*
7. Quickly add the 0.5 mL of gold particles from Step 4 at a constant rate into the Antibody Solution prepared in Step 5.

   Note: *Antibody concentration is now at 2.5 to 12.5 µg/mL OD1 gold particles.*
8. Immediately vortex mix for 10 seconds.[1, 3]
9. Incubate the tube using gentle rotation (e.g. 2-10 rpm) for 60 minutes at room temperature (20 – 25°C).[1, 3]

Preparing Blocker Solution

10. Prepare 10% (w/v) BSA Blocker Solution by adding 100 mg BSA to 1 mL of Conjugation Buffer.
11. Vortex mix thoroughly before use.

    Note: *Optimize the blocker type and concentration as required for the assay. Use freshly prepared Blocker Solution or pre-made solution stored at 2-8 °C for under a week.*

Blocking the Antibody-Conjugated Gold Nanoparticles

12. Add 0.1 mL of the Blocking Solution to the particle conjugate from Step 8 and vortex mix for 10 seconds.[1]
13. Incubate the tube using gentle rotation (e.g. 2-10 rpm) for 60 minutes at room temperature (20 – 25°C).[1]

Storage of Antibody-Conjugated Gold Nanoparticles

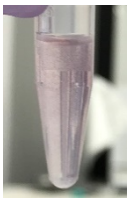
14. Centrifuge the conjugated particles at 2,000 x g for 10 minutes.
15. Carefully remove 1 mL of the supernatant, leaving about 0.1 mL of solution in the tube.[2]
16. Add 0.9 mL of Storage Buffer and re-suspend the pellet by pipetting or gentle vortex mixing for 10 seconds.
17. Centrifuge the particles at 2,000 x g for 10 minutes.
18. Carefully remove 0.95 mL of the supernatant.[2] Re-suspend the particles in the remaining solution by pipetting or gentle vortex mixing for 10 seconds.
19. The antibody-conjugated gold nanoparticles should now be ~50 µL with a concentration ≥ OD10.

    a. Check the conjugate OD with a UV-VIS spectrometer and adjust the final volume as necessary with Storage Buffer.

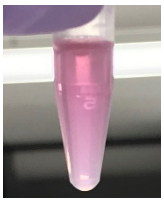
    b. Conjugates may be used immediately or may be stored at 2-8°C until required.[4, 5, 6]

Notes

1) Particle aggregation may occur during mixing and incubation of gold particles with Antibody Solution or Blocker Solution. This aggregation can be detected by a color change of dispersed particles from light pink or red to purple, gray, or colorless. The degree of color change is indicative of the severity of aggregation, and dark particulates may even be visible.



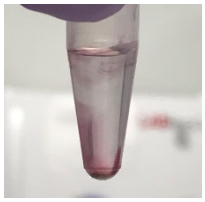
Aggregated (purple/colorless)



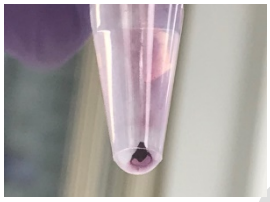
Stable (pink or red)

If such color change occurs, sonicate the solution in a bath sonicator for 5 to 30 minutes, until the solution returns to a pink color.[7]

2) Particle aggregation can be visually recognized after centrifugation by particles forming a very dense pellet or sticking to the tube wall as shown (aggregation is shown in both pictures).



Stuck to walls



Dense pellet

These particles may not re-disperse easily. To re-disperse, remove the supernatant, add the indicated amount of buffer, and sonicate the solution in a bath ultrasonicator for 5 to 30 minutes or until the pellet has re-dispersed and no particles stick to the walls.[7]

3) The two options below are recommended if aggregation is observed during conjugation:

   i. Increase antibody concentration during conjugation (e.g. from 6 µg/mL to 12 µg/mL) to maximize nanoparticle surface coverage.

   ii. Use co-conjugation for Step 5 by mixing BSA with antibody in the Conjugation Buffer. BSA may have a higher affinity for particle surface than the antibody, and high BSA concentrations may reduce assay sensitivity. Try a 1:1 ratio of BSA: antibody and optimize as needed with lower BSA concentrations (e.g. 1:2, 1:3).

4) OD is a measure of concentration defined as the peak absorbance of the conjugate (around 530 nm) with a 1 cm path length. OD can be adjusted following $C_1V_1 = C_2V_2$, where C = OD and V = volume.

5) Over time, nanoparticles will settle in the container leading to a concentration gradient as shown. The particles should fully re-disperse with gentle shaking or vortexing. Sonicate for 5 minutes if particles do not fully re-disperse.

Settled particles

6) If conjugated particles stick to the membrane during lateral flow tests (visible as a pink background), using an antigen-free chase buffer or sonicating the particles for 5 minutes after Steps 8, 12, 15, and/or 17 may help reduce this background.

7) Prolonged sonication or sonication at temperatures >30°C may impair antibody activity or assay performance. Sonicate in an ice bath or decrease sonication time if such reduction in activity is suspected or predicted.

Troubleshooting

Problem	Possible Causes	Optimization Solutions
Aggregation occurs during conjugation or blocking step [1, 2]	Insufficient washing of activated Au (Steps 1-4)	• Remove as much supernatant as possible after centrifugation in Steps 1-4 using sharp pipette tip
	High salt concentration in antibody buffer	• Desalt antibody before conjugation
	Incomplete coverage of nanoparticle surface	• Increase [Ab] or co-conjugate with BSA [3]
Low conjugate yield (<50 µL OD 10)	Aggregation	• See solutions for aggregation above
	Nanoparticles lost during wash step(s)	• Remove supernatant more carefully • Increase centrifugation time by 5-10 min
Low lateral flow intensity*	Aggregation – particles stuck in membrane	• See solutions for aggregation above
	Protein damaged during sonication	• Sonicate in ice bath • Reduce sonication time
	BSA blocking Ab attachment	• Reduce [BSA] during conjugation
High non-specific binding in lateral flow*	Too much BSA	• Lower BSA concentration in co-conjugation and/or blocker solution • Use BSA-free blocker solution (e.g. casein, FSG)

* Many factors affect lateral flow performance and these troubleshooting tips only pertain if conjugate is a factor

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