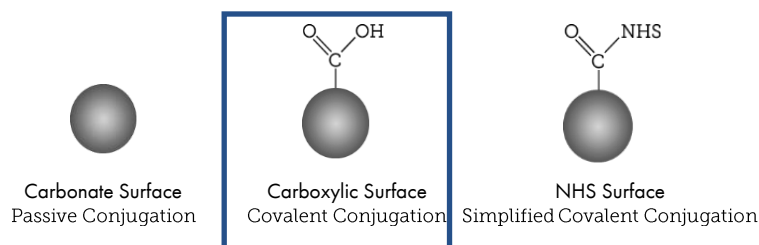


BioReady 40 nm Carboxylic Gold

Covalent Conjugation Protocol



1. INTRODUCTION

NanoComposix BioReady 40nm Carboxylic Gold (Au) can be conjugated to proteins through carbodiimide crosslinker chemistry. Covalent coupling of proteins (e.g. antibodies) to a gold nanoparticle surface yields robust and stable gold particle conjugates. The BioReady 40 nm Carboxylic Gold nanoparticles are functionalized with a tightly bound monolayer containing terminal carboxylic acid functional groups which can be activated through EDC/Sulfo-NHS chemistry to generate gold nanoparticle-antibody amide bonds.

For inquiries regarding custom conjugation, contact us at info@nanocomposix.com.

2. MATERIAL INFORMATION & STORAGE

BioReady Gold is supplied at OD 20 in water. The solution should be stored at 4 °C. Do not freeze. Thoroughly shake contents to disperse particles if settling occurs.

3. ADDITIONAL MATERIALS REQUIRED

- Antibody purification filters/columns
- DI H₂O
- Antibody purification buffer
 - 10 mM potassium phosphate, pH 7.4
- Reaction buffer
 - 5 mM potassium phosphate, 0.5% 20K MW PEG at pH 7.4
- EDC
- Sulfo-NHS
- Quencher
 - 50% (w/v) hydroxylamine
- Conjugate diluent
 - 0.1X PBS, 0.5% BSA, 0.05% Sodium Azide
- Centrifuge
- Standard microcentrifuge tubes*
- Vortex
- Rotator

*Tubes with specialized treatments (e.g. low-bind) or with residual plasticizer can have negative effects on the stability of the nanoparticles **during activation steps**.

Contact us for any questions regarding the recommended tubes.

EDC and Sulfo-NHS can be purchased from multiple suppliers such as Sigma-Aldrich or Thermo Fisher. Proper handling and storage of these reagents is **critical** for successful conjugations. The reagents should be stored with desiccant at -20 °C and 4 °C respectively (Refer to manufacturer Storage and Handling guidelines). **Ensure that the reagents are allowed to come to room temperature before opening vials.**

IMPORTANT: *The reaction buffers used when activating NHS esters and forming covalent bonds with your protein are important. The activation with EDC and Sulfo-NHS is most efficient at pH 5 (the colloidal solution will self adjust to this pH after reagent addition. Alternatively, colloid can be transferred into a low molarity MES buffer for activation step). The reaction of Sulfo-NHS activated particles with primary amines is most efficient at pH 7-7.5. For best results, perform conjugation step with suggested reaction buffer (or other amine-free, low-salt buffer in the pH 7-7.5 range). Performing the reaction at a higher pH drastically reduces the half-life of the NHS-ester. Once a stable conjugate is formed it can be transferred into the buffer of choice.*

4. ANTIBODY PREPARATION

The antibody for conjugation should be purified and adjusted to a concentration of 1 mg/mL into a low ionic strength buffer **free of additional proteins or free amines**. We recommend 10 mM potassium phosphate. Commercial antibodies may contain protein additives for stabilization (e.g. BSA), amines as a preservative (e.g. sodium azide), or amines in the buffer (e.g. Tris) that all need to be removed before covalent conjugation to nanoparticles. Antibodies can be purified from salt preservatives using spin columns or dialysis tubing with the appropriate molecular weight cut-off, and can be transferred into a non-amine containing buffer using the same mechanisms. Use appropriate columns to remove stabilizing proteins if needed. If Tris buffer is required to remove stabilizing proteins, the antibody will need to be purified a second time to transfer it to a suitable amine-free buffer.

5. ANTIBODY CONCENTRATION

For 40 nm Au, a typical antibody to gold ratio is 50 µg of antibody per 1 mL of 20 OD BioReady 40nm Carboxylic Au.

6. CONJUGATION PROTOCOL

This conjugation protocol is intended for 1 mL of OD 20 BioReady 40nm Carboxylic Au that will result in 1 mL of antibody-gold conjugate at OD 20. For larger or smaller volumes, scale proportionately.

IMPORTANT: Steps 1-6 should be completed immediately after solubilizing EDC/Sulfo-NHS to minimize hydrolysis of the Sulfo-NHS ester in water and enhance the efficacy of conjugation.

1. Prepare EDC and Sulfo-NHS at 10 mg/mL in H₂O immediately before conjugation steps.

HINT: Ensure the reagents are at room temperature before opening vials. Weigh out approximately 1-10 mg EDC and Sulfo-NHS each in individual microcentrifuge tubes. Just prior to conjugation, dissolve in the appropriate volume of H₂O to bring the concentration to 10 mg/mL

Example: Mass of EDC = 2.38 mg, add 238 μ L H₂O
Mass of Sulfo-NHS = 6.14 mg, add 614 μ L H₂O

2. Add 200 μ g EDC (20 μ L freshly prepared EDC at 10 mg/mL in H₂O) and 400 μ g Sulfo-NHS (40 μ L of freshly prepared Sulfo-NHS at 10 mg/mL in H₂O) to 1 mL of BioReady 40nm Carboxylic Gold.
3. Vortex solution and incubate at room temperature for 30 minutes while rotating.
4. Centrifuge at 3600 RCF for 10 minutes.
5. Carefully remove supernatant to remove any excess EDC/Sulfo-NHS and resuspend in 1 mL of **Reaction Buffer**. Vortex and/or sonicate (<30 seconds) to fully re-suspend particles.
6. Add antibody and vortex.
7. Incubate at room temperature for 1-2 hours while rotating (shorter or longer incubation times may yield better results).
8. After incubation, add 10 μ L of **Quencher** to deactivate any remaining active NHS-esters. Vortex and incubate at room temperature for 10 minutes while rotating.
9. Centrifuge at 3600 RCF for 10 minutes. Carefully remove supernatant and resuspend in 1 mL of reaction buffer. Vortex and/or sonicate to fully re-suspend conjugate.
10. Repeat centrifugation and re-suspension to remove any excess antibody.
11. Centrifuge again at 3600 RCF for 10 minutes, remove supernatant and bring volume up to 1 mL in **Conjugate Diluent**. Vortex and/or sonicate to fully re-suspend conjugate.
12. Store conjugate at 4°C. Do not freeze.

7. FREQUENTLY ASKED QUESTIONS

What is the shelf life of BioReady Gold nanoparticles?

We guarantee our BioReady particles for 6 months from date of purchase when our storage and handling guidelines are followed. Longer stability (1+ years) can be expected.

What is the shelf life of the nanoparticle-antibody conjugates?

The shelf life of the conjugate will depend on a number of factors including the antibody, the storage buffer components, and storage conditions. We recommend monitoring the stability of your conjugate over time for your specific application. A preservative (e.g. NaN₃) can be added to the storage buffer after conjugation. Optimal salt concentrations may differ between conjugates and can affect stability. Stabilizing proteins such as BSA can also help stabilize the conjugate. Store all conjugates at 4 °C.

Can I conjugate any type of antibody, or a protein that is not an antibody?

BioReady 40nm Carboxylic Gold can be used to covalently attach any proteins with free primary amines (-NH₂) by producing amide bonds.

Is there a test to confirm that my conjugates are functional?

Lateral flow assays are simple tests for evaluating conjugates. Contact us for preparation of custom test strips that can be used for the validation of your conjugate. For more information regarding lateral flow test format, refer to our handbook.

How do I optimize my conjugate?

Many variables can be adjusted to optimize the conjugate including the antibody/gold ratio, antibody incubation time, performing additional blocking steps, optimizing the conjugate diluent components, and evaluating the antibody purification buffer or reaction buffer. Lower antibody ratios may be useful for competitive assays if using conjugates for lateral flow format. When decreasing antibody loading, decreasing the antibody incubation time is recommended.

It is important to note that optimal conjugation procedures are antibody dependent; optimization techniques will differ from antibody to antibody.

What other particles are available for conjugation to proteins?

nanoComposix also offers BioReady 40nm Carbonate Gold for passive adsorption to proteins, BioReady 40nm NHS Gold for a simplified covalent conjugation procedure, and BioReady 150nm gold nanoshells that can yield up to a 20X increase in sensitivity for lateral flow assays.

8. ADDITIONAL RESOURCES

For more information on conjugation techniques and lateral flow assay development, please visit ncx.bz/br.

For technical assistance, please contact (858) 565-4227 x2 or email us at info@nanocomposix.com.

9. LIMITED USE LICENSE

NanoComposix conjugation reagents are offered for research purposes only and are not intended for human, therapeutic, or diagnostic use. For more information on the limit use license, visit ncx.bz/br.