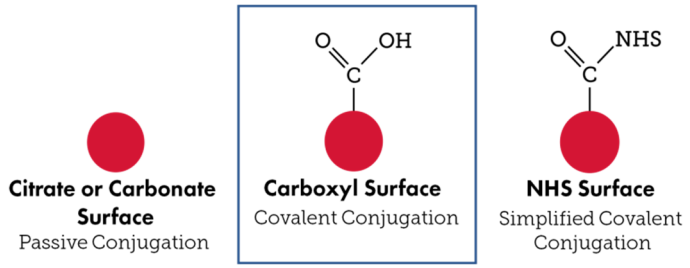
nanoComposix

BioReady™ 40 nm Carboxyl Gold

Covalent Conjugation Protocol

Product Number AUXR40



1. INTRODUCTION

Covalent coupling of proteins (e.g. antibodies) to a gold nanoparticle surface is a common method to yield robust and reproducible gold conjugates. nanoComposix's BioReady™ 40 nm Carboxyl Gold Spheres are functionalized with a tightly-bound monolayer containing terminal carboxylic acid functional groups. These particles can be easily activated through EDC/Sulfo-NHS (carbodiimide) chemistry to generate strong amide bonds between the gold nanoparticle and antibody, resulting in a stable and reliable conjugate.

This document provides a general guideline as a starting place for covalent conjugation, with recommendations to offer a strong chance of initial success. We recommend testing additional conditions to optimize the conjugation for your specific protein/antibody and application (see FAQs for more details).

Contact info@nanocomposix.com for inquiries regarding custom conjugation, technical support, or determining which nanoparticle is right for your application.

2. MATERIAL INFORMATION & STORAGE

BioReady™ 40 nm Carboxyl Gold is provided at OD 20 in water. Store at 4 °C. Do not freeze. Thoroughly shake or sonicate contents to disperse particles if settling occurs. Compatible microcentrifuge tubes are also provided and should be used during conjugation.

Proper handling and storage of EDC and Sulfo-NHS is critical for successful conjugations. These reagents can be purchased from a number of vendors and the manufacturer guidelines for handling and storage should be followed. EDC and Sulfo-NHS should be stored with desiccant at -20 °C and 4 °C respectively. Ensure that reagents are brought to room temperature before opening to avoid water condensation.

3. ADDITIONAL MATERIALS REQUIRED

- Buffer exchange/desalting column, *10 kDa MWCO*
- DI water
- Desalting buffer for antibody
  - *Recommended: 10 mM potassium phosphate, pH 7.4*
- Reaction buffer
  - *Recommended: 5 mM potassium phosphate, 0.5% 20K MW PEG at pH 7.4*
- EDC
- Sulfo-NHS
- Quencher
  - *Recommended: 50% (w/v) hydroxylamine*
- Conjugate diluent
  - *Recommended: 0.5X PBS, 0.5% BSA, 0.5% casein, 1% Tween 20, 0.05% Sodium Azide, pH 8*
- Centrifuge

  NOTE: The recommended centrifugation conditions in this protocol are based on a fixed-angle rotor centrifuge. If you are using a centrifuge with a swing out rotor (a.k.a. swinging bucket), you may need to adjust (likely increase) the centrifugation speeds to sufficiently pellet your nanoparticles.
- Centrifuge tubes

  NOTE: Tubes with specialized treatments (e.g. low-bind) or with residual plasticizer from the manufacture process can cause instability of the particles during activation steps. The tubes included with your BioReady™ Carboxyl Gold are compatible and should be used during conjugation. For more information, see:
  nanocomposix.com/pages/covalent-necessary #tubes
  - ***10 complimentary microcentrifuge tubes incldued with your purchase:*** *1.5 mL Labcon SuperSpin® tubes Cat# 3016-870-000*
  - *15 mL Labcon SuperClear® conical Cat #3131-335-028*
  - *50 mL Labcon SuperClear® conical Cat #3181-345-008*

  Available here: http://pipettipsandtubes.com/
- Vortex
- Rotator

IMPORTANT: *The reaction buffer used when activating NHS esters and forming covalent bonds with your protein is important. The activation with EDC and Sulfo-NHS is most efficient at pH 5, and the particle solution is designed to self-adjust to this pH after reagent addition. The conjugation of these activated particles with primary amines on the antibody is most efficient at pH 7-7.5. For best results, perform the conjugation step with the suggested reaction buffer or another 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 > 1 mg/mL in a low ionic strength buffer **free of additional proteins or free amines,** such as 10 mM potassium phosphate. Commercial antibodies may contain protein additives for stabilization (e.g. BSA), preservatives (e.g. sodium azide), or amines in the buffer (e.g. Tris) which 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 cutoff, and can be transferred into a non-amine-containing buffer using the same mechanisms. If Tris or another amine-containing buffer is used to elute the antibody from an affinity column during isolation from stabilizing proteins, the antibody will need to be buffer-exchanged into a suitable amine-free buffer.

5. ANTIBODY LOADING CONCENTRATION

For covalent conjugation to BioReady™ 40 nm Carboxyl Gold, a typical antibody-to-gold ratio is anywhere from 25-100 µg of antibody per 1 mL of gold at OD 20. For the initial conjugation described in this protocol, we recommend starting at 50 µg of antibody per 1 mL of gold at OD 20.

6. CONJUGATION PROTOCOL

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

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

> **IMPORTANT:** *Steps 2-5 should be completed immediately (within 5 minutes) after solubilizing EDC and Sulfo-NHS to minimize hydrolysis of the Sulfo-NHS ester in water and ensure conjugation efficiency.*

1. Thoroughly vortex and sonicate the BioReady™ 40 nm Carboxyl Gold Spheres to disperse and aliquot 1 mL of particles into one of the provided microcentrifuge tubes.
2. Prepare EDC and sulfo-NHS at 10 mg/mL in DI water immediately before conjugation steps.

   TIP: Ensure the reagents are at room temperature before opening vials. Weigh out approximately 1-10 mg EDC and sulfo-NHS in individual microcentrifuge tubes and record mass. Just prior to conjugation, dissolve in the DI water to bring the concentration to 10 mg/mL.

   Example: Mass of EDC = 4.3 mg, add 430 µL DI water
   Mass of sulfo-NHS = 6.1 mg, add 610 µL DI water

3. Add 200 µg EDC (20 µL freshly prepared EDC at 10 mg/mL) to 1 mL of BioReady™ 40 nm Carboxyl Gold Spheres.
4. Add 400 µg sulfo-NHS (40 µL of freshly prepared sulfo-NHS at 10 mg/mL) to the 1 mL BioReady™ 40 nm Carboxyl Gold Spheres.
5. Vortex solution and incubate at room temperature for 30 minutes while rotating.
6. Centrifuge at 3800 RCF for 10 minutes†.
7. Carefully remove supernatant without disturbing the pellet to remove any excess EDC/sulfo-NHS and resuspend pelleted nanoparticles with 1 mL **reaction buffer**. Vortex and/or sonicate (<30 seconds) to fully re-suspend particles.
8. Repeat step 6-7 to wash particles of any excess EDC/NHS.
9. Add 50 µg antibody and vortex.
10. Incubate at room temperature for 1 hour while rotating (shorter or longer incubation times may yield better results).
11. After incubation, add 10 µL of **quencher** to deactivate any remaining active NHS-esters. Vortex and incubate at room temperature for 10 minutes while rotating.
12. Centrifuge at 3800 RCF for 10 minutes†. Carefully remove supernatant without disturbing the pellet and resuspend pellet with 1 mL of **reaction buffer** (wash #1). Vortex and/or sonicate to fully resuspend conjugate.
13. Repeat centrifugation† and resuspension with reaction buffer to remove any excess antibody (wash #2).
14. Centrifuge again at 3800 RCF for 10 minutes†. Carefully remove supernatant and bring volume of pellet up to 1 mL in **conjugate diluent**. Vortex and/or sonicate to fully resuspend conjugate.
15. Store conjugate at 4°C**. Do not freeze.**

† The gold should form a pellet after centrifugation with little to no color remaining in the supernatant. If supernatant remains colored, you may need to adjust your centrifugation conditions (e.g. increase centrifugation speed or time). Insufficient centrifugation will result in lower yield.

7. FREQUENTLY ASKED QUESTIONS

What is the shelf life of BioReady™ Gold nanoparticles?
We guarantee our BioReady™ particles for 12 months from date of delivery when our storage and handling guidelines are followed. Longer stability (> 2 years) can be expected.

What is the shelf life of the conjugates?
The shelf life of the conjugate depends on a number of factors including the antibody, storage buffer, and storage conditions. Monitor the stability of your conjugate over time for your specific application. A preservative can be added to the storage buffer **after** conjugation. Optimal salt concentrations may differ between conjugates and can affect stability. Proteins such as BSA can also help stabilize the conjugate. Store all conjugates at 4°C.

Can I conjugate any type of antibody or protein?
BioReady™ Carboxyl Gold can be used to covalently attach any proteins with free primary amines ($-NH_2$) 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, 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, blocking steps, conjugate diluent, and reaction buffer. Lower

antibody ratios may be required for competitive lateral flow assays. When decreasing the antibody loading, it is also recommended to decrease the antibody incubation time. Optimal incubation time can be as short as 5 minutes.

What can I do to improve conjugate release and flow on a lateral flow test?

We recommend optimizing the amount of surfactants, proteins, and adding other components in the conjugate diluent as needed. Addition of sucrose and/or trehalose can also help with the release of the conjugate after drying down into the conjugate pad. Changing the pH or the buffers of the final conjugate diluent may help stabilize larger particle conjugates. Selection of the pad materials and appropriate treatment can also aid in the release and flow of the conjugate.

8. ADDITIONAL RESOURCES

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

Watch our webinars and video tutorials related to bioconjugation and lateral flow at ncx.bz/kb.

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

9. PRODUCT USE

NanoComposix conjugation reagents are intended for research use only unless otherwise noted on the Certificate of Analysis (CoA) or Certificate of Conformance (CoC) for the product. cGMP-compliant versions of many BioReady products are available upon request. Please contact us at info@nanocomposix.com for additional information and pricing.