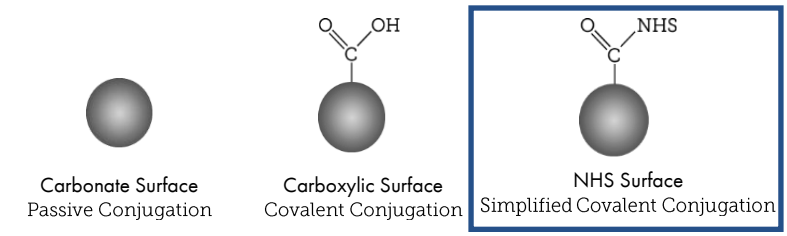
nanoComposix

BioReady 40 nm NHS Gold

Covalent Conjugation Protocol



1. INTRODUCTION

NanoComposix BioReady 40 nm NHS Gold (Au) can be covalently conjugated to primary amines (-NH_2) of proteins in a simplified procedure. Covalent coupling of proteins (e.g. antibodies) to a gold nanoparticle surface yields robust and reliable gold conjugates. The BioReady 40 nm NHS Gold nanoparticles are surface functionalized with an active NHS ester to generate gold nanoparticle-antibody amide bonds, eliminating the need for the user to perform the intermediary EDC/NHS chemistry steps.

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

2. MATERIAL INFORMATION & STORAGE

BioReady 40 nm NHS Gold nanoparticles are supplied as a lyophilized dried powder in aliquots of either 50 µL or 500 µL upon re-dispersion. The gold powder should be stored with desiccant packets (as shipped) at -20 °C and reconstituted just before before use. When reconstituted, the particles are monodisperse and at OD 20 at the specified aliquot volume. Remaining kit components should be stored according to label.

3. ADDITIONAL KIT COMPONENTS

- Antibody Purification Filters
  - *Amicon® Ultra 0.5 mL Filters 10 kDa*
- 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*
- Quencher
  - *5% (w/v) hydroxylamine*
- Conjugate Diluent
  - *0.1X PBS, 0.5% BSA*
- Other Materials/Equipment Required:
  - Centrifuge
  - Microcentrifuge tubes
  - Vortexer
  - Rotator

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 40 nm NHS Au.

6. CONJUGATION PROTOCOL

This conjugation protocol is intended for **50 µL** of OD 20 BioReady 40 nm NHS Au that will result in 50 µL of antibody-gold conjugate at OD 20. For larger volumes, scale proportionately. ***Conjugations are carried out at approximately 1 mL scale to ensure adequate mixing. The conjugate will concentrated to 20 OD in the final step.**

1. Remove one reaction aliquot of BioReady 40 nm NHS Gold from freezer and bring to room temperature (~20 minutes). Store remaining aliquots with provided desiccant at -20 °C until use.
2. Prepare your reconstitution buffer by combining 1 mL of **Reaction Buffer** with 2.5 µg purified antibody
3. Reconstitute NHS Gold by adding the reconstitution buffer with antibody prepared in step 2. Gently pipette up and down to reconstitute, or bath sonicate (<30 seconds).
4. Incubate at room temperature for 1-2 hours while rotating (shorter or longer incubation times may yield better results).
5. After incubation, add 10 µL of **Quencher** to deactivate any remaining active NHS-esters.
6. Transfer conjugate to a microcentrifuge tube.
7. 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.
8. Repeat centrifugation and re-suspension to remove any excess antibody.

9. Centrifuge again at 3600 RCF for 10 minutes. Carefully remove supernatant and bring up to final volume of 50 µL in **Conjugate Diluent** for a conjugate of OD 20. Vortex and/or sonicate to fully re-suspend conjugate.
10. Store conjugate at 4°C. **Do not freeze.**

7. FREQUENTLY ASKED QUESTIONS

What is the shelf life 40 nm NHS Gold particles?
NHS esters on the surface of our BioReady-NHS gold are stable in the dried format provided, but will hydrolyze within hours or minutes of being exposed to moisture (depending on water-content and pH of the reaction solution). The NHS-esters on the gold surface have a half-life of approximately 1-2 hours at pH 7, and only 10 minutes at pH 8. Best results are obtained when NHS-activated gold is used promptly upon resuspension for reaction to the amine-containing targets. With proper storage and handling (desiccated, at -20°C) BioReady gold NHS particles are stable for at least 6 months.

What is the difference between the BioReady 40 nm NHS Gold and the BioReady 40 nm Au Carboxylic?
Both BioReady 40 nm Gold materials (NHS surface and Carboxylic surface) can be covalently conjugated to amines of antibodies or peptides through carbodiimide crosslinker chemistry. The NHS surface is a lyophilized powder that has an activated NHS surface on the particles. To conjugate the Carboxylic Au, it is necessary to perform the intermediary steps of activating the carboxylic groups to semi-stable amine-reactive NHS ester groups through EDC/Sulfo-NHS chemistry.

What are the advantages of using the NHS Au vs. the Carboxylic Au?
The NHS Au is supplied in a format that allows for rapid and simple conjugation to antibodies. However, the gold solution must be used immediately upon resuspension and is only available in small scale format. The carboxylic surface requires the end user to perform the intermediary EDC/Sulfo-NHS chemistry, but can be used for larger scale conjugations and can be used for full optimization of the antibody-gold conjugate.

Can I conjugate any type of antibody, or a protein that is not an antibody?
BioReady 40 nm Carboxylic and NHS 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 40 nm Carbonate Gold for passive adsorption to proteins, BioReady 40 nm Carboxylic Gold for a covalent conjugation, 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

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