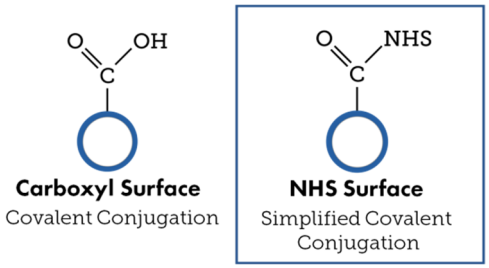
nanoComposix

BioReady™ 150 nm NHS Gold Nanoshells

Covalent Conjugation Protocol

Product Number GSNR150



1. INTRODUCTION

NanoComposix BioReady™ 150 nm NHS Gold Nanoshells can be covalently conjugated to primary amines (-NH_2) in proteins using a simplified format. Covalent coupling of proteins (e.g. antibodies) to the gold nanoparticle surface yields robust and reliable gold conjugates. The surface of BioReady™ 150 nm NHS Gold Nanoshells are functionalized with an active NHS ester, allowing amide bonds to form directly between the activated gold nanoparticle and your antibody. This eliminates the need to perform multi-step EDC/NHS chemistry normally required for covalent conjugation, significantly reducing the time and processing required for conjugation.

The BioReady™ 150 nm NHS Gold Nanoshell kit provides a convenient method for screening antibodies. Once an initial screen has been performed, nanoComposix offers nanoparticles that are more cost effective and flexible for optimizing your conjugate to achieve the desired sensitivity or function. We offer products that are suitable for passive adsorption (BioReady™ Bare Gold) or covalent conjugation (BioReady™ Carboxyl Gold) for increased sensitivity in a scalable format.

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

2. MATERIAL INFORMATION & STORAGE

BioReady™ 150 nm NHS Gold Nanoshells are supplied as a lyophilized powder in small or large aliquots (100 µL and 1 mL, respectively, when reconstituted to 20 OD). The gold powder should be stored with desiccant packets (as shipped) at -20 °C and reconstituted immediately before use. When reconstituted, the particles are activated for immediate conjugation to your antibody (attached via lysine residues). The resulting covalent conjugates are more stable than those prepared by passive adsorption methods. Unlike passive adsorption, the conjugation procedure does not depend on the antibody's isoelectric point. All antibodies are able to efficiently bind at a single fixed pH, eliminating the need for experimentally determining the optimal pH during conjugate development.

Remaining kit components should be stored according to their labels.

3. ADDITIONAL KIT COMPONENTS

- Antibody purification buffer
  - *10 mM potassium phosphate, pH 7.4*
- Reaction buffer
  - *5 mM potassium phosphate, 0.5% 20K MW PEG, pH 7.4*
- Quencher
  - *50% (w/v) hydroxylamine*
- Conjugate diluent
  - *0.5X PBS, 0.5% BSA, 0.5% Casein, 0.5% Tween 20, 0.05% Sodium Azide pH 8*
- Antibody purification filters *(available separately)*
  - *Amicon® Ultra 0.5 mL Filters - 10 kDa MW cutoff*

    A purified antibody is required for conjugation. You may use your own purification filters or purchase the nanoComposix reaction kit "with Spin Filters" and we'll include filters in your kit for your convenience.
- Additional materials/equipment required:
  - *Centrifuge*
  - *Centrifuge tubes*
  - *Vortexer*
  - *Rotator*

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 is possible. 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 these same methods. 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 purified a second time to transfer it to a suitable amine-free buffer.

Antibody purification into an amine-free buffer is different from affinity purification of the antibody during development. Even a small concentration of sodium azide (NaN_3) will interfere with the conjugation.

5. CONJUGATION PROTOCOL

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

NOTE: *This protocol is intended for use for small and large reaction kits. For both reaction sizes, the conjugation is carried out at a 1 mL scale to ensure adequate mixing. Conjugates will be concentrated in the final steps by centrifugation and resuspension to a final OD of 20. Refer to Table 1 for suggested reagent volumes for each size reaction kit.*

Table 1. Suggested reagent volumes for **small** and **large** reaction kits.

	Small Reaction (100 µL at 20 OD)	**Large Reaction** (1 mL at 20 OD)
Reaction buffer volume	1 mL (used twice; 2 mL total)	1 mL (used twice; 2 mL total)
Purified antibody	2 µg	20 µg
Volume of quencher	5 µL	10 µL
Final volume for 20 OD conjugate	100 µL	1 mL

1. Remove **small** or **large** reaction aliquot of BioReady™ 150 nm NHS Gold Nanoshells from freezer and bring to room temperature (~20 minutes). Store remaining aliquots with provided desiccant at -20 °C until use.

   IMPORTANT: *Steps 2-3 should be completed immediately after reconstituting NHS gold powder to minimize hydrolysis of the Sulfo-NHS ester in water and enhance the efficiency of conjugation.*

2. Open reaction aliquot and *carefully* add 1 mL of **reaction buffer**. Gently pipette up and down to resuspend. Bath sonicate <30 seconds if needed.
3. Add **antibody** according to **Table 1**.
4. Vortex to mix.
5. Incubate at room temperature for 1 hour while rotating (shorter or longer incubation times may yield better results).
6. After incubation, add volume of **quencher** specified in **Table 1** to deactivate any remaining active NHS-esters.
7. Incubate for 10 minutes while rotating.
8. Transfer conjugate to a microcentrifuge tube.
9. Centrifuge at 2000 RCF for 5 minutes. Carefully remove supernatant and resuspend pellet with 1 mL of **reaction buffer**. Vortex and/or bath sonicate to fully resuspend conjugate.
10. Repeat centrifugation and resuspension (Step # 9) to remove any excess antibody.
11. Centrifuge again at 2000 RCF for 5 minutes. Carefully remove supernatant and bring pellet up to final volume specified in **Table 1** with **conjugate diluent** for a resulting conjugate at OD 20. Vortex and/or sonicate to fully resuspend conjugate.
12. Store conjugate at 4°C. **Do not freeze.**

6. FREQUENTLY ASKED QUESTIONS

What is the shelf life 150 nm NHS Gold Nanoshells?
The NHS esters on the surface of our BioReady™ NHS Gold are stable in the dried format provided but will hydrolyze within minutes to hours of being exposed to moisture depending on the 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 immediately after resuspension for reaction with the amine-containing targets. With proper storage and handling (desiccated, at -20°C) BioReady™ gold NHS particles are stable for at least 6 months from the date of delivery.

What is the difference between the BioReady™ NHS Gold and the BioReady™ Carboxyl Gold?
Both BioReady™ Gold materials (NHS surface and Carboxyl surface) can be covalently conjugated to amines of antibodies or peptides through carbodiimide activation chemistry. The final conjugate is the same. The NHS gold is a lyophilized powder that has an activated NHS surface on the particles. To conjugate the Carboxyl gold, it is necessary to perform the intermediate steps of activating the carboxyl groups to semi-stable amine-reactive NHS ester groups through EDC/Sulfo-NHS chemistry.

What are the advantages of using NHS vs. Carboxyl gold?
The NHS gold 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 aliquots. The carboxyl surface requires the end user to perform the intermediate EDC/Sulfo-NHS chemistry, but can be used for larger scale conjugations and can be used for full optimization of the antibody-gold conjugate.

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 to validate your conjugate. For more information regarding lateral flow, refer to our handbook at ncx.bz/hb.

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 components, antibody purification buffer, 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.

7. 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 x2 or email us at info@nanocomposix.com.

8. 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.