nanoComposix

BioReady™ Streptavidin Gold Conjugation Protocol

Product Number AUIR40

INTRODUCTION

NanoComposix BioReady™ Streptavidin Gold can be conjugated to biotinylated proteins through affinity interactions. The avidin-biotin interaction is one of the strongest non-covalent affinity interactions. BioReady™ gold nanoparticles with Streptavidin are prepared by covalently attaching the tetrameric protein to the surface of the functionalized nanoparticles, with excellent retention of biotin-binding activity.

For inquiries regarding custom conjugation, technical support, or determining which gold product is right for you, contact *info@nanocomposix.com*

MATERIAL INFORMATION & STORAGE

BioReady™ Streptavidin-Gold is provided at OD 10 in 1× PBS, 0.5% BSA, and 0.05% azide.

Store the particles at 4° C. Do not freeze. Thoroughly shake contents to disperse particles if settling occurs.

ADDITIONAL MATERIALS

- **Biotinylated reagent for attachment**
- **Biotin quantitation kit/HABA assay** (optional)
- **Rotator**
- **Biotin** (optional)
- **Wash/storage buffer** (1× PBS with 0.5% 20K MW PEG, or another buffer of choice)
- **Centrifuge tubes**
- **Centrifuge**

STREPTAVIDIN-BIOTIN CONJUGATION RATIO

For conjugation of biotinylated reagent to BioReady™ Streptavidin Gold, the optimal ratio will depend on how much biotins are available. In many cases, there may be multiple biotin moieties per protein. To determine the amount of biotin on your protein, run a biotin quantitation/HABA assay.

CONJUGATION PROTOCOL

The following procedure provides some general guidance for conjugating 1 mL of OD 10 BioReady™ Streptavidin Gold to biotinylated antibody. For larger volumes, centrifugation speed and time may need to be adjusted.

Conjugation procedures may vary depending on the biotinylated reagents and other conditions specific to the different types of application.

Note: Prior to conjugation with biotin, Streptavidin Gold solution can be exchanged into another buffer of choice by centrifuging the particles, remove the supernatant, then resuspend in the desired buffer.

1. Aliquot BioReady™ Streptavidin Gold in an Eppendorf tube or other reaction vessel.
2. To this solution, add biotinylated IgG. The amount of biotinylated reagent added will have to be optimized for each antibody or application.
3. Incubate at room temperature for 30 minutes with end-over-end rotation or rocking. Incubation time can also be adjusted to optimize performance.

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4. To prevent potential cross-linking of particles, quench any remaining avidin sites with an excess of biotin (optional).
5. Centrifuge the conjugate at the specified speed and time in the table, carefully remove the supernatant, and resuspend in the wash/storage buffer. Repeat this wash step 3× to remove any excess biotinylated IgG in the solution.

	Centrifugation speed (RCF)	Time (minutes)
40 nm Gold Spheres	3600	10
150 nm Gold Nanoshells	2000	5

6. For final resuspension, bring the conjugate to the desired storage concentration.
7. Store conjugate at 4° C**. Do not freeze.**

FREQUENTLY ASKED QUESTIONS

What is the shelf life of the nanoparticles?

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

What is the shelf life of the conjugates?

The shelf life of the conjugate will depend on many factors including the antibody stability, storage buffer components, and storage conditions. We recommend monitoring the stability of your conjugate over time for your specific application. A preservative (e.g. NaN_3) can be added to the storage buffer **after** conjugation. 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 protein?

BioReady™ Streptavidin Gold can be used to covalently attach any proteins conjugated to biotin moieties.

How is the streptavidin attached to the surface of the gold particle?

The nanoparticles are functionalized with a tightly bound monolayer containing terminal carboxylic acid functional groups. The functional groups are activated using standard EDC/Sulfo-NHS chemistry to generate covalent gold nanoparticle-Streptavidin amide bonds

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

Lateral flow assays are simple and effective tests for evaluating conjugates. Contact us for preparation of custom test strips that can be used for the validation of your conjugate.

How do I optimize my conjugate?

Many variables can be adjusted to optimize the conjugate including the biotinylated protein/gold ratio, blocking steps, and conjugate diluent components. 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?

For covalent conjugates, we recommend optimizing the amount of surfactants and other components in the conjugate diluent such as Tween 20 (1–2%), BSA (0.5–1%), or additional reagents as needed. Increasing the pH of the final conjugate diluent may help stabilize larger particle conjugates.

ADDITIONAL RESOURCES

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

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

For technical assistance, contact (858) 565-4227 x 2 or email us at *info@nanocomposix.com*

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