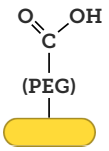
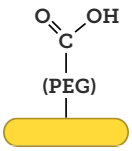
nanoComposix

NanoXact™ Carboxyl Gold Nanorod Covalent Conjugation

Product Numbers GRXH660 & GRXH800



Peak λ 660 nm
40 nm × 15 nm



Peak λ 800 nm
55 nm × 15 nm

INTRODUCTION

NanoComposix NanoXact™ Gold Nanorods are surface plasmon resonant particles with peak resonances that are spectrally aligned to standard laser wavelengths. Given their potential use for therapeutic and diagnostic applications, it is often desirable to conjugate gold nanorods to antibodies, proteins, aptamers, and other biological molecules.

NanoComposix NanoXact™ PEG-Carboxyl Gold Nanorods offer a convenient means of covalent conjugation to free amines in biological molecules through EDC/sulfo-NHS chemistry. In the EDC/NHS activation chemistry, EDC is used to activate the carboxyl group on the surface of nanorods to create a crosslinker. The resulting intermediate can bind to primary amines on the biomolecule but is unstable and susceptible to hydrolysis. Sulfo-NHS is added with EDC to create a more stable amine-reactive intermediate and facilitate binding to primary amines.

For inquiries regarding custom conjugations, technical support, or determining which nanoparticle is right for your application, contact *info@nanocomposix.com*

MATERIAL INFORMATION & STORAGE

NanoXact™ PEG-Carboxyl Gold Nanorods are provided at OD 50 in water. Store at 2–8 °C. Do not freeze. Thoroughly shake contents to disperse particles if settling occurs.

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's 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.

ADDITIONAL MATERIALS

- **DI water**
- **EDC**
- **Sulfo-NHS**
- **Reaction buffer**
  E.g. 0.01X PBS w/ 0.5% PEG
- **Quencher**
  E.g. 50% (w/v) hydroxylamine
- **Conjugate diluent**
  E.g. 0.01X PBS w/ 0.5% PEG, or BSA in 4 mM borate buffer w/ 1.5% azide
- **Centrifuge tubes (**pipettipsandtubes.com)
  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
- **Centrifuge**
- **Vortex**
- **Rotator**

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CONJUGATION GUIDE

The following steps provide general guidelines for conjugating NanoXact™ PEG-Carboxyl Gold Nanorods to free amines in proteins. Optimal conjugation procedures will vary depending on the protein, the reagents used, and other conditions specific to the application.

This conjugation guide uses 300 µL of OD 50 NanoXact™ PEG-Carboxyl Gold Nanorods and will yield 1 mL of antibody-gold conjugate at OD 15. To alter the volume or concentration, scale proportionately.

1. Tubes with specialized treatments (e.g. low-bind), or with residual plasticizer from the manufacturing process, can cause instability of the particles during activation steps. If using tubes other than those specified in this guide, thoroughly rinse tubes with isopropyl alcohol and DI water before use. Aliquot 300 µL of NanoXact™ PEG-Carboxyl Gold Nanorods into an Eppendorf tube or other appropriate reaction vessel. Dilute with 700 µL DI water to achieve a total volume of 1 mL. Shake to mix.

Important: *The EDC/NHS steps should be completed immediately after solubilizing EDC and Sulfo-NHS to minimize hydrolysis of the Sulfo-NHS ester in water and enhance the efficiency of conjugation.*

2. Prepare EDC and Sulfo-NHS solutions at 10 mg/mL in DI water immediately prior to use.
3. Add 20 µL of EDC solution to diluted gold nanorods.
4. Add 40 µL Sulfo-NHS to gold nanorods immediately after EDC addition.
5. Sonicate and/or vortex briefly before placing on a rotating incubator for 30 mins at room temperature.
6. Centrifuge the conjugate. We recommend starting at 5000 RPM for ~10 minutes. Spinning rate and duration can be increased as needed. Monitor the conjugate for signs of aggregation between cycles.
7. Carefully remove supernatant containing excess EDC/NHS and resuspend pellet in 1 mL of buffer. Sonicate and/or vortex to ensure particles are fully resuspended.
8. Add antibody or protein and vortex to mix.
9. Incubate for a minimum of 4 hours while rotating.
10. After incubation, add 10 µL of quencher to deactivate remaining NHS ester/EDC groups. Incubate for an additional 5 minutes.
11. Centrifuge, collect pellet, and remove supernatant. [Repeat successful spinning conditions from step 6.] Resuspend in 1 mL of buffer. Vortex/sonicate to mix.
12. Repeat step 11 one more time to ensure removal of excess antibody.
13. Centrifuge the conjugate for a final cycle and bring to the desired storage concentration using conjugate diluent of choice.
14. Store conjugate at 4 °C. Do not freeze.

FREQUENTLY ASKED QUESTIONS

What is the shelf life of NanoXact™ Gold Nanorods?

We guarantee our NanoXact™ Gold Nanorods for 6 months from date of delivery when our storage and handling guidelines are followed. Longer stability (> 1 year) 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 protein, storage buffer, and storage conditions. Monitor 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. 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?

NanoXact™ PEG-Carboxyl Gold Nanorods 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 components, purification buffer, and reaction buffer. Lower antibody or protein ratios may be required for competitive lateral flow assays. When decreasing the antibody loading, it is also recommended to decrease antibody incubation time.

ADDITIONAL RESOURCES

NanoComposix offers a comprehensive set of custom conjugation services, including end-to-end development, optimization of existing procedures, and contract manufacturing of conjugates at liter scales.

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 or email us at *info@nanocomposix.com*

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

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