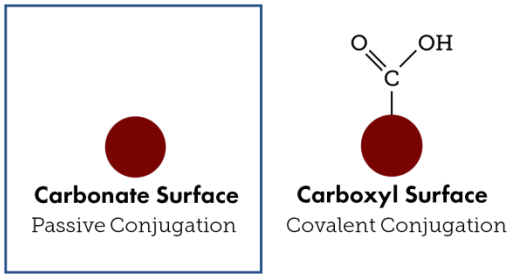
nanoComposix

BioReady™ 80 nm Bare Gold

Passive Conjugation Protocol

Product Number AUKR80



1. INTRODUCTION

NanoComposix BioReady™ 80 nm Bare Gold can be conjugated to proteins using passive adsorption (physisorption). The mechanism of adsorption is based on Van der Waals and other interactions between the proteins (e.g. antibodies) and the surface of the particles. The resulting forces between the antibody and the nanoparticle are influenced by the nanoparticle surface and the coupling environment. Passive conjugation efficacy is dependent on the isoelectric point of the antibody. Since the isoelectric point will differ for each antibody, the optimal pH for passive conjugation should be determined experimentally by titrating the gold to a range of different pH values (e.g. increments of 0.2 from pH 7-9) prior to adding the antibody. The resulting conjugates can be compared by observing the colloidal stability (e.g. color change, change in UV-vis spectra or DLS measurement) and the conjugate performance in your specific application.

NanoComposix also offers 80 nm gold and additional nanoparticle variants that have been surface functionalized for covalent conjugation to antibodies and do not require a pH titration. Covalent conjugations can be more stable and can produce conjugates with improved batch-to-batch reproducibility and increased sensitivity in some assays. Performance enhancements for passive vs. covalent binding will depend on the position of available primary amines on each antibody. For more information please visit ncx.bz/br.

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

2. MATERIAL INFORMATION & STORAGE

BioReady™ 80 nm Bare Gold is provided at an optical density (OD) of 5 at λ_{max} (~540 nm), and is available with a carbonate surface. Colloidal gold nanoparticles are suspended in DI water with a weakly associated surface to stabilize the particles. In the presence of protein, the surface is rapidly displaced to passively conjugate the protein.

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

3. ADDITIONAL MATERIALS

- Antibody purification filters/columns
- Potassium phosphate monobasic (optional)
  - *To titrate pH down*
- Potassium phosphate dibasic (optional)
  - *To titrate pH up*
- Salt solution (10% w/v NaCl) (optional)
- DI H_2O
- Conjugate block buffer
  - *25 mM borate, 10% BSA*
- Conjugate diluent
  - *25 mM borate, 1% BSA*
- Antibody purification buffer
  - *10 mM potassium phosphate, pH 7.4*
- Centrifuge
- Beaker or glass test tubes (>15 mL)
- Parafilm (optional)
- Stir bar & stir plate (if using a beaker)
- Vortexer & rotator (if using glass test tubes)

4. ANTIBODY PREPARATION

The antibody for conjugation should be purified into a low ionic strength buffer **free of additional proteins or salt components**, such as 10mM potassium phosphate. Commercial antibodies may contain protein additives for stabilization (e.g. BSA), salt as a preservative (e.g. sodium azide), or salt in the storage buffer (e.g. PBS) that need to be removed before passive adsorption of antibodies to nanoparticles. Antibodies can be purified into a non-salt containing buffer using spin columns or dialysis tubing with the appropriate molecular weight cut-off. We recommend storing purified antibodies at a concentration ≥ 1 mg/mL. It is important to note that antibody stability varies and you should always refer to data sheet provided by the antibody supplier for proper storage and handling.

5. ANTIBODY CONCENTRATION

For passive adsorption to 80 nm gold, a typical antibody-to-gold ratio is 15 µg of antibody per 1 mL of gold at OD 5. Adjusting the amount of antibody may improve conjugation results. For information regarding the optimization of the antibody concentration, refer to **section 7** after the conjugation protocol.

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 intended for **4 mL of OD 5** BioReady™ 80 nm Bare Gold that will result in 1 mL of antibody-gold conjugate at OD 20. For larger or smaller volumes, scale proportionately.

1. Rinse all glassware with DI H_2O to ensure materials are free of impurities/contaminants.
2. Aliquot 4 mL gold OD 5 into a glass beaker with stir bar or to a glass test tube (>15 mL tube size).

3. Rapidly add 60 µg of purified antibody to the gold solution (refer to **section 7** for optimizing antibody concentration).

   NOTE: *Best results are seen with rapid addition of the antibody. Ensure that the solution is stirring rapidly if using a beaker, or add the antibody solution to the gold while vortexing in a glass test tube.*

4. Cover beaker or test tube with parafilm and incubate at room temperature for 30 minutes while stirring/rotating.
5. Block conjugate by adding 0.5 mL of conjugate block buffer (10% BSA). Vortex/mix the solution.
6. Incubate at room temperature for 30 minutes while stirring/rotating.
7. Centrifuge at 3800 RCF for 5 minutes.
8. Carefully remove supernatant and resuspend with conjugate diluent to 1 mL final volume for an OD 20 conjugate. Store at 4˚C. **Do not freeze.**

7. ANTIBODY CONCENTRATION OPTIMIZATION

It may be beneficial to adjust the antibody-to-gold ratio. The following procedure can be used to determine the minimum amount of antibody needed to protect the conjugate from aggregation:

1. Dilute gold to OD 1 with water for 2 mL final volume.
2. (e.g. 0.4 mL 5 OD gold + 1.6 mL water)
3. Aliquot 200 µL of gold into eight tubes.
4. Add 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.6 µg of antibody to each of the gold aliquots.

   HINT: *To improve pipetting accuracy, prepare a working dilution at 100 µg/mL in antibody purification buffer immediately prior to conjugation.*

5. Incubate at room temperature for 30 minutes while rotating.
6. Add 20 µL of 10% NaCl solution to each tube and incubate 20 minutes.
7. Observe the samples for loss of colloidal stability, indicated by a change from red to purple/gray.

Figure 1. Stability study to determine the optimal antibody concentration for passive conjugation. Figure above shows a concentration of ≥ 0.4 µg of this antibody per 200 µL of gold OD 1 is required to prepare a stable conjugate.

8. Determine the lowest antibody concentration that provides colloidal stability **(Figure 1)**.
9. Divide this number by the volume (0.2 mL) to determine the appropriate amount of antibody to add to each mL of 80 nm gold at OD 1 (e.g. 0.6 µg /0.2 mL = 3 µg antibody/1 mL of OD 1 gold) then multiply the resulting number by 5 to determine the appropriate amount of antibody to add to each mL of 80 nm gold at OD 5 (e.g. 3 µg antibody/1 OD·mL x 5 OD = 15 µg antibody per 5 OD·mL)

8. 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™ Bare Gold can be used for passive adsorption of antibodies or other proteins/peptides to the surface. BioReady™ Bare Gold is provided between pH 7-8.5, and should be adjusted to the optimal pH for your specific antibody to achieve best results. The pH of the Bare Gold should be slightly higher (~0.2 pH units) than isoelectric point (pI) of the protein. Monoclonal antibodies typically have a more well-defined pI and changing the pH of the gold solution may result in more efficient binding. For other types of proteins or peptides, the pI range is greater which requires a broader range of pH titrations to maximize binding efficacy.

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.

How do I optimize my conjugate?

Many variables can be adjusted to optimize the conjugate including the pH of conjugation, the incubation time during conjugation/blocking, and the blocking buffer components. Optimal conjugation procedures are antibody dependent; optimization techniques will differ from antibody to antibody.

What other particles are available for conjugation?

NanoComposix offers a BioReady™ product line with a wide range of particle sizes and surfaces to choose from. BioReady™ 150 nm gold nanoshells can yield up to a 20X increase in sensitivity for many lateral flow assays.

9. 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.

10. LIMITED USE LICENSE

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/br.