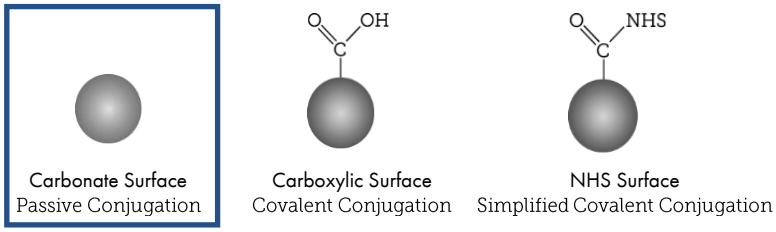
nanoComposix

BioReady 40nm Carbonate Gold

Passive Conjugation Protocol



1. INTRODUCTION

NanoComposix BioReady 40 nm Carbonate Gold (Au) can be conjugated to proteins through passive adsorption (physisorption). The mechanism of adsorption is based on van der Waals 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. The BioReady 40nm Carbonate Au is provided at a pH of approximately 8.0-8.5, which is suitable for conjugation to many IgG antibodies. A pH titration can be performed to optimize the pH of conjugation for each individual antibody.

At nanoComposix, we also offer 40 nm gold and 150 nm gold nanoshells that have been surface functionalized to allow for covalent conjugation to antibodies that require no pH titration. Covalent conjugations are typically more stable and can produce conjugates with improved batch-to-batch reproducibility and increased sensitivity in certain assays.

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

2. MATERIAL INFORMATION & STORAGE

BioReady 40 nm Carbonate Gold is provided at Optical Density (OD) 4.2 +/- 0.3 at a pH of ~8.5. The solution should be stored at 4 °C. Do not freeze. This conjugation protocol is intended for **10 mL of OD 1** BioReady Carbonate Au. Dilute Au to OD 1 with DI H_2O before starting protocol or scale accordingly. Thoroughly shake contents to disperse particles if settling occurs.

To dilute to OD 1, use the following equation and enter the initial OD (OD_1= starting OD provided on the Certificate of Analysis), the final OD (OD_2= 1), and the desired final volume (V_2).

$OD_1V_1=OD_2V_2$

3. ADDITIONAL MATERIALS REQUIRED

- Antibody purification filters/columns
- 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 (>15mL)
- 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 and adjusted to a concentration of 1 mg/mL into a low ionic strength buffer **free of additional proteins or salt components**. We recommend 10 mM 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 from salt preservatives using spin columns or dialysis tubing with the appropriate molecular weight cut-off, and can be transferred into a non-salt containing buffer using the same mechanisms. Use appropriate columns to remove stabilizing proteins if required.

5. ANTIBODY CONCENTRATION

For passive adsorption to 40nm gold, a typical antibody-to-gold ratio is 5 µg of antibody per 1 mL of Au at OD 1. Increasing or decreasing 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

This conjugation protocol is intended for **10 mL of OD 1** BioReady 40 nm Carbonate Au that will result in 1 mL of antibody-gold conjugate at OD 10. For larger or smaller volumes, scale proportionately.

1. Dilute Au with H_2O to OD 1 for 10 mL final volume.
2. Rinse all glassware with DI H_2O to ensure materials are free of impurities/contaminants.
3. Aliquot 10 mL Au OD 1 into a glass beaker with stir bar or to a glass test tube (>15 mL tube size).
4. Rapidly add the antibody to the Au solution (50 µg* antibody per 10 mL of Au OD 1).

NOTE: *A pH titration may improve the efficiency and sensitivity of the conjugate.*

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.*

5. Incubate at room temperature for 30 minutes while stirring/rotating.
6. Block conjugate by adding 100 µL of conjugate block buffer (10% BSA). Vortex/mix the solution.
7. Incubate at room temperature for 30 minutes while stirring/rotating.
8. Centrifuge at 3600 RCF for 10 minutes.
9. Carefully remove supernatant and resuspend with conjugate diluent to 1 mL final volume for an OD 10 conjugate. Store at 4 °C. Do not freeze.

7. ANTIBODY CONCENTRATION OPTIMIZATION*

To determine the minimum amount of antibody needed to protect the conjugate from aggregation, perform the following procedure:

1. Dilute Au to OD 1 with H_2O for 2 mL final volume.
2. Aliquot 200 µL of Au into eight 1-2 mL tubes.
3. Add 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.6 µg of antibody to each of the Au aliquots.
   a. For more accurate antibody addition, prepare a working dilution of the antibody to 100 µg/mL in antibody purification buffer prior to conjugation.
4. Incubate at room temperature while rotating for 30 minutes.
5. Add 20 µL of 10% NaCl solution to each tube and incubate 20 minutes.
6. Observe the samples for loss of colloidal stability, indicated by a change from red to purple/grey.
7. Determine the lowest antibody concentration that provides colloidal stability **(Figure 1)**.

Figure1. 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 Au OD 1 is required for a stable conjugate.

8. Multiply this number by 5 to determine the appropriate µg of antibody to add to each mL of 40nm Au at OD 1 (i.e. 0.4 µg X 5= 2 µg antibody/mL of OD 1 Au).

8. FREQUENTLY ASKED QUESTIONS

What is the shelf life of the nanoparticles?
We guarantee our BioReady particles for 6 months from date of purchase when our storage & handling guidelines are followed. Longer stability (1+ years) can be expected.

What is the shelf life of the particle conjugates?
The shelf life of the conjugate will depend on many factors including the antibody, the 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 a protein that is not an antibody?
BioReady Au can be used for passive adsorption of antibodies or other proteins/peptides to the surface. The pH of the BioReady Au is ~8.5, and should be adjusted to the appropriate pH for your specific conjugation to achieve optimal results. Typically, the pH of the Au should be slightly higher (~0.2 pH units) than pI of the protein. Monoclonal antibodies typically have a more defined pI and changing the pH of the Au solution may result in more effective 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 also offers BioReady 40nm Carboxylic gold and NHS surface gold for covalent conjugation, as well as BioReady 150nm gold nanoshells that can yield up to a 20X increase in sensitivity for lateral flow assays.

9. 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.

10. LIMITED USE LICENSE

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