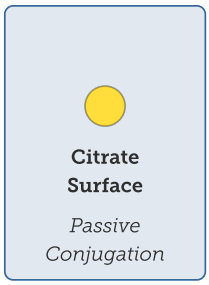
nanoComposix

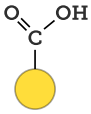
BioReady™ 40 nm Bare Gold · Passive Conjugation Protocol

Product Number AUCR40



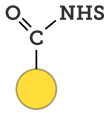
Citrate Surface

Passive Conjugation



Carboxyl Surface

Covalent Conjugation



NHS Surface

Simplified Covalent Conjugation

The following procedure demonstrates the process of pH titration to conjugate an IgG antibody to **250 µL of OD 20** BioReady™ 40 nm Bare Gold. A **pH range at 7, 8, and 9** will be evaluated to determine the optimal point for passive binding. If desired, 0.5 or 0.2 (or any other increment) of pH point can also be investigated. A larger range of pH from 6–10 can also be evaluated if necessary with the appropriate buffers (see Further Optimization).

Since the isoelectric point will differ for each protein used, the optimal pH for passive conjugation should be determined empirically.

It's important to note that this protocol provides a general guideline only. Optimal conjugation procedures are protein-dependent and optimization techniques will differ for each protein/assay.

MATERIALS NEEDED

- **NanoComposix BioReady™ 40 nm Bare Gold**
- **Protein buffer exchange/desalting columns**
- **Protein purification buffer** (e.g. 5–10 mM potassium phosphate, pH 7.4)
- **100 mM buffers for titration with pH range 7–9**
- **≥ 1 mg/mL protein**
- **10% sodium chloride solution**
- **Conjugate block buffer** (e.g. 10 mM potassium phosphate or borate, 10% BSA, pH 7.5–8.5)
- **Conjugate diluent** (e.g. 10 mM potassium phosphate or borate, 1% BSA, pH 7.5–8.5)
- **1.5–2 mL centrifuge tubes**
- **Vortexer, rotator, centrifuge, and bath sonicator**

PROTEIN PREPARATION

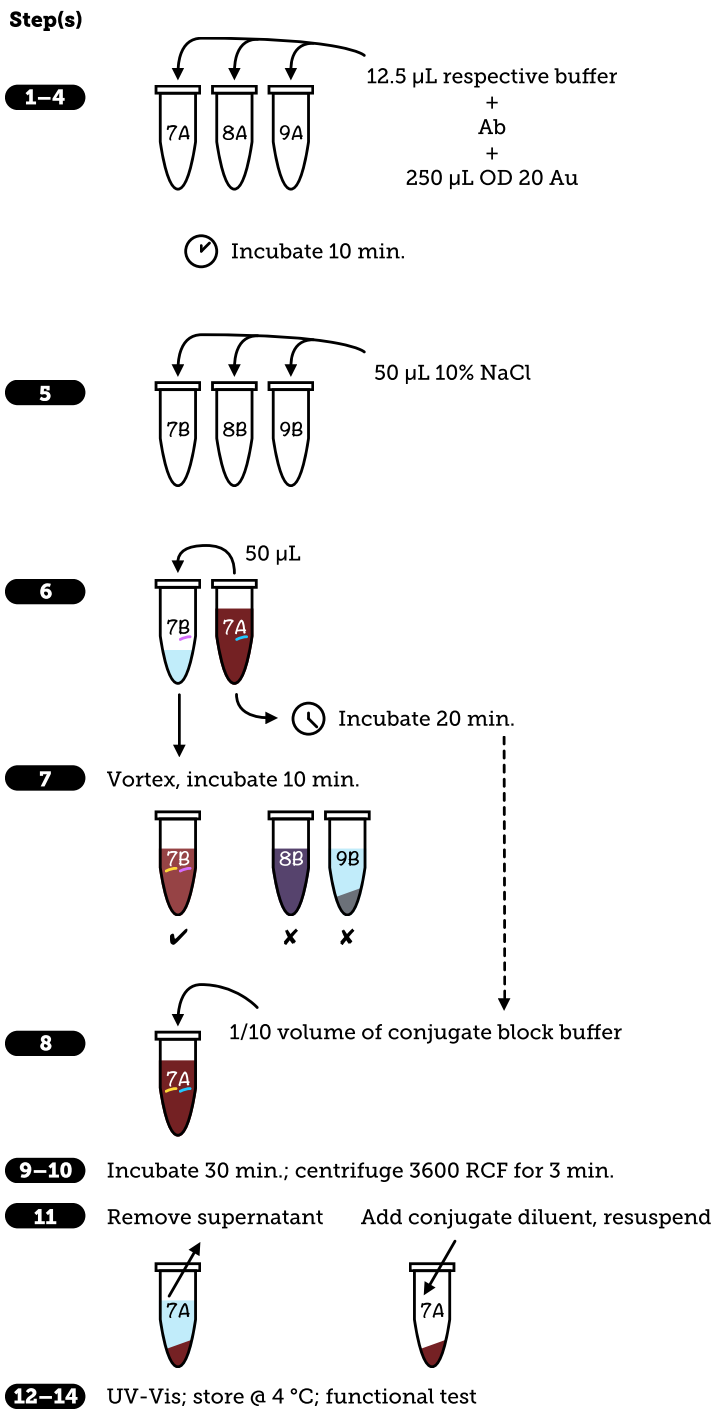
The protein for conjugation can be purified into a buffer **free of additional proteins or salt components**. Some commercial products, antibodies for example, may contain additives for stabilization (e.g. BSA) or preservatives (e.g. sodium azide) that need to be removed to increase the efficiency of passive adsorption. Protein can be purified into the appropriate buffer using spin columns or dialysis tubing with the appropriate molecular weight cut-off. **We recommend using purified protein for conjugation at a concentration ≥ 1 mg/mL**. Refer to the data sheet(s) provided by the supplier for proper storage and handling.

CONJUGATION LOADING

For passive adsorption to 40 nm gold, a typical loading range can be anywhere from 25–160 µg of protein per mL of OD 20 gold. However, the optimal loading can also vary outside of this range depending on the protein used and the assay's performance. For information regarding the optimization of the protein loading, refer to the Further Optimization section.

Without knowing the optimal loading for the antibody, a loading of 80 µg Ab per mL of OD 20 gold (or **20 ug Ab per 250 µL of gold**) is chosen for this procedure as a starting point to ensure at least sufficient coating for the pH titration process. The optimal loading can be evaluated after a pH point has been selected (see how to optimize the antibody loading in the Further Optimization section).

PH TITRATION FLOW CHART



PROTOCOL

1. Prepare a set of 3 eppi tubes. Label each of the tubes 7A, 8A, and 9A to correspond with each pH point.
2. Add 12.5 µL of each 100 mM buffer to its respectively labeled tube. **The volume of buffer added should be around 1/20 of the total working volume of gold**.
3. Add 20 µg of antibody into each of the tubes containing the buffer from step #2.
4. Add 250 µL of OD 20 gold to each of the tubes from step #3. Vortex and incubate on the rotator for 10 minutes.
5. Aliquot 50 µL of 10% NaCl into another separate set of 3 eppi tubes. Label each of the tubes 7B, 8B, and 9B.
6. After about 10 minutes of incubation for the "A" tubes (buffer + Ab + gold):
   a. Pipette 50 µL of the conjugate from each "A" tube into each respective "B" tube containing NaCl.
   b. Leave the remaining "A" tube volume to incubate for an additional 20 minutes.
7. Vortex the NaCl + conjugate mixture in the "B" tubes. Incubate on rotator for about 10 minutes and observe any color change:
   a. Stable conjugate: the gold maintains its red color.
   b. Unstable conjugate: the gold becomes purple, gray, clear, or precipitated.

   Move forward with the pH condition(s) that produced a stable conjugate.
8. After the "A" tubes from step 6b have incubated for a total of 30 minutes, select the tubes that correlated to the stable "B" tubes. Add 1/10 volume of conjugate block buffer so that the final BSA concentration in each "A" tube is around 1%.
9. Incubate for 30 minutes on rotator.
10. Centrifuge the conjugate at 3600 RCF for 3 minutes.
11. Carefully remove the supernatant and resuspend with conjugate diluent buffer to the target OD (e.g. 200 µL final volume for OD 20). Vortex and bath sonicate if needed to fully resuspend the conjugate.
12. Perform UV-Vis reading and check for the final OD.
13. Store conjugate at 4°C until use. **Do not freeze.**
14. Functionally test the selected pH condition(s) on the final application (e.g. lateral flow strips).

FURTHER OPTIMIZATION

Other pH points can be evaluated by preparing the appropriate buffers at the pH of interest for screening. As an option for further optimization, smaller increments around the selected pH can be done to evaluate the optimal pH point. For example, if both pH 7 and pH 8 resulted in stable conjugates and performed well with the functional testing, a 0.3 pH increment can be done to evaluate pH 6.8, 7.1, 7.4, 7.7, 8.0, and 8.3 to further optimize this process. The following buffers can be used for the evaluation:

Buffer	Buffering Capacity (pH)
MES	5.5–6.7
HEPES/potassium phosphate	6.8–8
Borate	8–10
Carbonate-bicarbonate	9–10

Once an optimal pH point has been determined for the conjugation, the protein to gold ratio can be varied to determine the optimal protein/antibody loading. At the previously determined optimal pH, vary the amount of antibody added to the buffer in step #3. We recommend evaluating a range between 20–200 µg of protein loading to find the protein loading that yields a stable conjugate and has the highest performance in the functional assay. For best results, **the volume of protein added to the conjugation should not exceed 10% of the total gold volume** as this may dilute the final buffer concentration and cause the pH to drift slightly (concentrate the protein to above 1 mg/mL if necessary).

Other parameters that should be optimized for the assay include protein incubation time, blocking time, raw materials, and chemistry formulations (block buffer, conjugate diluent/storage buffer, running buffer, any pad treatment buffers if needed, etc.). If there are substantial changes to the other components of the assay during development, re-optimization of the conjugate may be required.

FREQUENTLY ASKED QUESTIONS

Can I perform the conjugation at a different OD?

Yes, this general procedure is applicable to conjugate nanoComposix BioReady™ 40 nm Bare Gold at any OD from 1 to 20. To perform the conjugation at a lower OD, dilute the gold solution to the desired OD in 0.2 mM citrate:

1. Prepare 20 mM citrate pH 5.0.
2. Add 1/100 volume of the 20 mM citrate buffer to the OD 20 gold solution so the OD 20 gold is in a 0.2 mM final concentration of citrate.
3. Separately, prepare a stock solution of 0.2 mM citrate by diluting the 20 mM citrate 1/100 in diH_2O.
4. Combine the OD 20 gold from step #2 with the 0.2 mM citrate in water in step #3 to dilute the gold down to the desired OD.
5. Follow the pH titration protocol above to do the conjugation.

Can I use potassium carbonate to do the pH titration?

Yes, with gold that has been diluted to 5 OD or lower with 0.2 mM citrate, the pH can be titrated with potassium carbonate to screen different pH's.

Can I conjugate any type of antibody or protein?

BioReady™ Bare Gold can be used for passive adsorption of antibodies and other proteins or peptides to the surface. BioReady™ Bare Gold should be adjusted to the optimal pH for your specific protein to achieve best results.

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.

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/protein 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. Proclin or NaN_3) can be added to the storage buffer **after** conjugation. The addition of other proteins such as BSA can also help stabilize the conjugate. Store all conjugates at 4 °C.

What other particles are available for conjugation?

NanoComposix also offers BioReady™ 40 nm Carboxyl and NHS Gold for covalent conjugation, and BioReady™ 150 nm Carboxyl Gold Nanoshells that can yield up to a 20× increase in sensitivity for certain lateral flow assays.

SUPPLEMENTAL INFORMATION

NanoComposix BioReady™ 40 nm Bare Gold is conjugated to proteins using passive adsorption (a.k.a. physisorption). The mechanism of adsorption is based on Van der Waals forces and other interactions between the proteins (e.g. antibodies) and the surface of the particles. The binding of the protein to the nanoparticle is influenced by the nanoparticle surface and the solution environment, as well as the isoelectric point of the protein.

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

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

Version 2.3 · Published October 2020