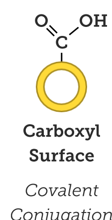
BioReady™ 150 nm Citrate Gold Nanoshells

Passive Conjugation Protocol



The following procedure demonstrates the process of pH titration to conjugate an IgG antibody to **250 μL of OD 20** BioReady™ 150 nm Citrate Gold Nanoshells. A **pH range at 7, 8, and 9** will be initially evaluated for passive binding. 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™ 150 nm Citrate Gold Nanoshells, Product Number: GSCR150**
- **Protein buffer exchange/desalting columns**
- **Protein purification buffer**
  Example: 5–10 mM potassium phosphate, pH 7.4
- **100 mM buffers for titration with pH range 7–9.** See Reaction Buffer Examples list
- **≥ 1 mg/mL protein**
- **Conjugate block buffer**
  Examples:
  5mM potassium phosphate, 5% BSA, pH 7.5-8.5
  5 mM sodium borate, 10% BSA, pH 7.5–8.5
- **Conjugate diluent**
  Example: 0.5x PBS, 0.5% Casein, 0.5% BSA, 1% Tween-20, 0.05% Azide
- **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.

REACTION BUFFER EXAMPLES

Below are some common buffers for use in passive conjugation. Prepare at 100 mM and titrate to the desired pH.

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

Version 1.2 · Published June 2022

CONJUGATION LOADING

For passive adsorption to 150 nm gold nanoshells, a typical loading range can be anywhere from 10–100 µg of protein per mL of OD 20 gold nanoshells. 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 30 µg Ab per mL of OD 20 gold nanoshells (or **7.5 ug Ab per 250 µL of gold nanoshells**) 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.

PROTOCOL

1. Prepare a set of 3 eppi tubes. Label each of the tubes 7, 8, and 9 to correspond with each pH point tested.
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 7.5 µg of antibody into each of the tubes containing the buffer from step #2.
4. Add 250 µL of OD 20 gold nanoshells to each of the tubes from step #3. Vortex and incubate on the rotator for 30 minutes.
5. After incubation, observe any changes in the opacity of the solutions. A stable conjugate will remain opaque, while unstable conjugates become more transparent. See **Image 1** below for reference. Hold the tubes up to a light source if it is difficult to determine which are opaque.

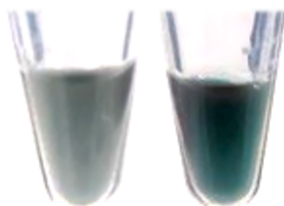


Image 1: Unstable conjugate (left), stable conjugate (right)

6. Move forward with the pH condition(s) that produced a stable conjugate.
7. Add conjugate block buffer equal to 1/10 of the total working volume of gold so that the final BSA concentration in each tube is around 1%.
8. Incubate for 30 minutes on rotator.
9. Centrifuge the conjugate at 2000 RCF for 2 minutes.
10. 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 briefly bath sonicate if needed to fully resuspend the conjugate.
11. Perform UV-Vis reading and check for the final OD.
12. Store conjugate at 4°C until use. **Do not freeze.**
13. Functionally test the conjugates for each stable pH condition on the final application (e.g. lateral flow strips).
14. Note which pH point(s) yielded the best performing conjugate(s) and repeat steps 1-13, testing additional pH points in a smaller increments to determine the optimal pH condition. See pH Titration under **Further Optimization** for additional details.
15. Repeat conjugations using only the best performing pH condition from step 14 while evaluating higher and lower antibody loadings to determine the optimal antibody loading condition. See Antibody Loading Titration under **Further Optimization** for additional details.

FURTHER OPTIMIZATION

Note on Salt Stability and Salt Test

Passive conjugations sometimes utilize a salt test to quickly screen pH or antibody loading conditions. In this test, salt is added to conjugates 10 minutes after the initial incubation begins. If any pH or antibody loading conditions become unstable during this test, they are eliminated from further work. If a salt test is performed with BioReady™ 150 nm Citrate Gold Nanoshells, start by trying an 0.375 – 0.5% NaCl solution and adjust the concentration based on results. BioReady™ 150 nm Citrate Gold nanoshells are more sensitive to salt concentration than traditional colloidal gold. Use caution when working with high salt buffers and reduce the salt concentration of the buffer or consider switching to another buffer if instability is noted.

pH Titration

Certain proteins may work well within a small or large pH range. Following the initial pH titration performed in steps 1-13 of the protocol, additional pH points can be tested to further optimize the conjugation. This additional testing may use 0.2, 0.5, or other small pH increments, as needed:

1. Note which pH point(s) from step #13 performed best following functional testing.
    a. If a range of pH's were optimal, test pH increments within this range.
    b. If a single pH point was optimal, test pH points in small increments both above and below this point.
2. Repeat steps 1-14 of the protocol and continue testing until no notable performance difference is observed between the pH points that are evaluated.

In case there are multiple pH points that yield a stable conjugate, choose the pH point most similar to the pH of the antibody storage solution, if possible. For example, if the conjugate is stable at pH 7.0–8.4, and the antibody is stored at pH 7.4, move forward with 7.4.

Antibody Loading Titration

A loading of 7.5 µg Ab per 250 µL of OD 20 BioReady™ 150 nm Citrate Gold Nanoshells is used as a starting point, but this amount may be higher than what's needed for the conjugation.

Once an optimal pH point has been determined, the protein to gold nanoshell ratio can be varied to determine the optimal loading using the following protocol as an example:

1. Prepare a set of 4 eppi tubes and pipette 12.5 µL of the optimal 100mM buffer determined in step 14 of the protocol into each.
2. Add 1 µg, 2.5 µg, 5 µg, and 10 µg of antibody into each tube.
3. Proceed with steps 4–13 of the protocol.
4. Move forward with the lowest amount of Ab required to produce optimal conjugates.

Depending n the type or size of protein used, a range between 10–100 µg per 1 mL OD 20 BioReady™ 150 nm Citrate Gold Nanoshells can be evaluated to find the loading with the highest performance in the functional assay.

Other Considerations

Additional parameters that should be optimized for the assay can 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

What happens if the conjugates crashed at all pH points in step # 5?

1. Proceed through the addition of blocking buffer and incubation in steps # 7 and 8 and then check the conjugates again. Some conjugates may appear unstable prior to blocking, but then recover after blocking.
2. Try testing additional pH's outside the range evaluated initially or test additional pH points at smaller increments between the pH's evaluated initially.
3. Increase the antibody loading.
4. Decrease the amount of NaCl used.
5. Check the storage buffer of the Ab or protein. There may be preservatives, salts, or other additives that may interfere with the conjugation. If this is the case, purify or buffer-exchange the protein to remove these additives before conjugation.
6. Check the pH of each conjugate (buffer + protein + gold solution) to make sure the pH is around the pH of the buffers added. Buffer exchange the protein or Ab into a lower molarity solution (5–10 mM) if needed.

Can I perform the conjugation at a different OD?

Yes, this general procedure is applicable to conjugate BioReady™ 150 nm Citrate Gold Nanoshells 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 nanoshell solution so the OD 20 gold nanoshells are 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 nanoshells from step #2 with the 0.2 mM citrate in water in step #3 to dilute the gold nanoshells down to the desired OD.

How can I troubleshoot false positive or non-specific binding?

1. Evaluate different blocking time (1h, 2h, or overnight).
2. Shorten the antibody/protein incubation time.
3. Change the buffer formulation for blocking buffer, conjugate diluent, or running buffer (vary the pH's, concentrations, and components in the buffers).
4. Try using a different grade of membrane, different type of conjugate pad, or use less conjugate per strip.

How can I improve signal intensity?

1. Increase the amount of conjugate per strip.
2. Use a slower speed membrane.
3. Evaluate different buffer formulations for conjugate diluent, block, or running buffer.
4. Increase the antibody/protein incubation time.

Can I conjugate any type of antibody or protein?

BioReady™ 150 nm Citrate Gold Nanoshells can be used for passive adsorption of antibodies and other proteins or peptides to the surface. The gold should be adjusted to the optimal conjugation 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 conjugates?

The shelf life of the conjugate will depend on many factors, including the antibody/protein stability, storage buffer components, storage conditions, and the conjugate pad. The storage buffer/conjugate diluent provided with the kit is not a universal solution for all proteins/conjugates. Different formulation and optimization may be required for each assay.

We recommend monitoring the stability of your conjugate over time for your specific application. Store all conjugates at 4 °C.

What other particles are available for conjugation?

NanoComposix also offers BioReady™ 150 nm Carboxyl Gold Nanoshells for covalent conjugation as well as BioReady™ 40 Citrate Gold Nanospheres and BioReady™ 80 nm Citrate Gold Nanospheres for passive conjugation.

ADDITIONAL RESOURCES

The topics covered in this procedure are meant to serve as a general guideline. Some assays may require more extensive optimization or a different approach to obtain the best performance. For more information on conjugation techniques and contract 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*