nanoComposix

BioReady™ Passive Conjugation Kit Protocol

Product Number PCKR

The Passive Conjugation Kit is designed to help conjugate antibodies and other proteins to the surface of our BioReady™ 40 nm and 80 nm Bare Citrate Gold Spheres. Since the isoelectric point will differ for each protein used, the optimal pH for passive adsorption needs to be determined to find the optimal binding condition, which can be quickly screened with the buffers provided with the kit.

The following protocol outlines the steps to conjugate **250 µL of OD 20** BioReady™ 40 nm and 80 nm Citrate Gold to an IgG antibody. The buffers provided in the conjugation kit cover a **pH range from 6.0 to 9.7**, but another range outside of 6 and 10 can also be tested if desired (see *Further Optimization*).

It is important to note that this protocol and the buffers provided serve only as a general guideline. Optimal conjugation procedures are protein-dependent; optimization techniques and buffer formulations will differ for each protein/assay.

KIT COMPONENTS

- **NanoComposix BioReady™ 40 nm and 80 nm Bare Citrate Gold**
- **100 mM buffers for pH titration**
- **10% sodium chloride solution**
- **Conjugate block buffer**
- **Conjugate diluent**

ADDITIONAL RECOMMENDED MATERIALS

- **≥ 1 mg/mL protein for conjugation**
- **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 and 80 nm gold, a typical loading range can be anywhere from 10–120 µg of protein per mL of OD 20 gold.

Without knowing the optimal loading for the antibody, a loading of 80 µg Ab per mL of 40 nm OD 20 gold, or 40 µg Ab per mL of 80 nm OD 20 gold (**20 ug or 10 ug Ab per 250 µL of gold**) is chosen for this procedure as a starting point.

A lower or higher Ab loading can be evaluated after a pH point has been selected (see how to optimize the antibody loading in the *Further Optimization* section).

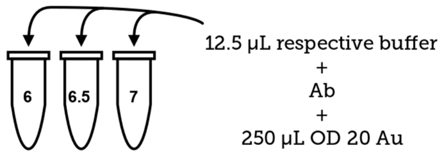
SALT TEST WITH NACL

10% NaCl is used to test the stability of the conjugate during the process of pH titration or antibody loading study. Samples with NaCl added are used for visual inspection to identify the most stable condition(s) only, and should be discarded when done.

PH TITRATION FLOW CHART

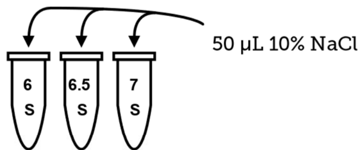
Step(s)

1–4



Incubate 10 min.

5



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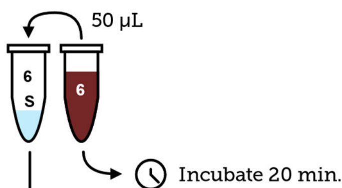
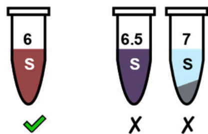


7 Vortex, incubate 10 min.



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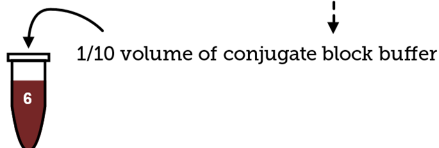

9–10 Incubate 30 min.; centrifuge 3600 RCF for 3 min.

11 Remove supernatant Add conjugate diluent, resuspend

12–14 UV-Vis; store @ 4 °C; functional test

PROTOCOL

1. Prepare a set of 8 eppi tubes. Label the tubes with the pH point (6, 6.5, 7, 7.5, etc.).
   Printed labels are provided in the kit for convenience.
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 volume of gold**.
3. Add the following amount of antibody into each of the tubes containing the buffer from step #2:

Gold	Volume	OD	Ab to add
40 nm	250 µL	20	20 µg
80 nm	250 µL	20	10 µg

4. Add 250 µL of OD 20 gold to each of the tubes from step #3. Vortex and incubate on the shaker for 10 minutes.
5. While waiting, aliquot 50 µL of 10% NaCl into another separate set of 8 eppi tubes. Label the tubes with the pH points and the letter “S” (6S, 6.5S, 7S, etc.).
   Printed labels are provided in the kit for convenience.
6. After about 10 minutes of incubation for the conjugate from step 4 (buffer + Ab + gold):
   a. Pipette 50 µL of the conjugate into each respective “S” tube containing NaCl.
   b. Leave the remaining conjugate volume to incubate for an additional 20 minutes.
7. Vortex the NaCl + conjugate mixture. 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.
   Continue working with only the pH condition(s) that produced a stable conjugate.
8. After the tubes from step 6b have incubated for a total of 30 minutes, select the tubes that correlated to the stable “S” tubes. Add 1/10 volume of conjugate block buffer so that the final BSA concentration is around 0.5%.
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

Antibody Loading Titration

A loading of 20 µg Ab per 250 µL of 40 nm, OD 20 gold (or 10 µg for 80 nm gold) 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 ratio can be varied to determine the optimal loading using the following protocol with 40 nm gold as an example:

1. Exchange the antibody into a 5-10 mM buffer at the optimal pH as determined previously (or, use the buffer provided in the kit to titrate to the optimal pH). If the optimal pH for binding is the same as the antibody storage solution, no buffer exchange is needed.
2. Prepare a set of 4 eppi tubes and pipette 250 µL of 40 nm OD 20 gold into each tube.
3. Add 2.5 µg, 5 µg, 10 µg, and 20 µg of antibody into each tube containing the gold.
4. Incubate for 5–10 minutes on a shaker.
5. Prepare another set of 4 eppi tubes with 50 µL of NaCl in each tube.
6. Add 50 µL of each conjugate to the tubes containing NaCl and observe any color changes.
7. The conditions with insufficient antibody coating would crash immediately.
8. Move forward with the lowest amount of Ab required to produce a stable conjugate.

Depending on the type or size of protein used, a range between 10–120 µg 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

How can I choose the right pH?

Certain proteins may work well within a small or large pH range. In case there are multiple pH points that yield a stable conjugate, choose the pH 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 in 1X PBS pH 7.4, move forward with 7.4. This way, the antibody can be added directly to gold for conjugation without any additional buffer to adjust the pH.

Another more accurate way to figure out the optimal pH is by using the final assay/application to evaluate the conjugate performance.

What happens if the conjugates crashed at all pH points during the salt test?

1. Try a couple other pH's outside the range provided.
2. Increase the antibody loading.
3. Decrease the amount of NaCl used.
4. 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.
5. 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 nanoComposix BioReady™ Bare Citrate 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.

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 antibody/protein loading or 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 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 Citrate Gold 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 nanoparticles?

We guarantee our BioReady™ particles for 1 year 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, 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™ 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.

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*

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