Procedure for ligand with determined pI:

1. Allow kit reagents and samples to warm to room temperature, 20-25°C.
2. Mix by inverting the bottle of Naked Gold Sol (NG30-B009 or NG40-B009) to re-suspend any settled particles. Quickly dispense 0.5 mL of Naked Gold Sol into a test tube.
3. Use Table 1 on Page 3 to add the proper volumes of Buffers A and B, or C and D to the test tube. The pH selected should be slightly higher than the pI of the ligand to be conjugated. Mix thoroughly with a low speed vortex.
4. Add 12 µL of the solution containing ligand at 2 mg/mL to the test tube when using NG30-B009. Use 8 µL of the solution containing ligand at 2 mg/mL to the test tube when using NG40-B009. Mix carefully with a low speed vortex.
5. Allow the reaction to continue for a total of 30 minutes.
6. Stop the reaction by adding 50 µL of BSA Blocking Solution (BLK-002). Mix carefully and incubate for 5 minutes at room temperature. **NOTE:** In some conjugates that exhibit non-specific reactivity, it is often useful to allow the BSA Blocking Solution to react for up to an additional 16 hours to ensure complete coating of the conjugate.

The gold conjugate is now ready for use in a rapid assay at an optimal volume of 2 - 10 µL per test.

NOTE: This reaction may be scaled as needed to produce a larger volume of conjugate.

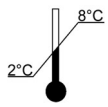
Drying of Gold Conjugate:

To effectively dry a gold conjugate, add 0.1 mL of Conjugate Drying Buffer (CDB-002) for every 0.9 mL of gold conjugate (1.0 mL total volume). Mix thoroughly. Apply conjugate solution gradually and evenly to either glass fiber or polyester ribbon.

Place ribbons in a forced-air (37°C ± 2°C) oven or incubator for >1 hour to dry thoroughly. Store desiccated.

WARRANTY:

REF NGIB34-B018



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BioAssay Works®

Gold-in-a-Box™ Conjugation Kit, 30 nm & 40 nm

Catalog No: REF NGIB34-B018

For Research Use Only

INTENDED USE:

The Gold-in-a-Box conjugation kit is for preparing antibody or protein gold conjugates to two different sizes of gold nanoparticles. For optimal binding of the ligand while retaining a high degree of specific activity, the pH of the gold sol must be adjusted to slightly above its isoelectric point. This kit allows quick determination of the optimal pH and coating range for your ligand. The resultant stabilized gold conjugates can be used in lateral flow assays without requiring additional optimization.

Each kit contains sufficient components to conjugate approximately 0.4 mg of protein to 30 nm gold and 0.25 mg of protein to 40 nm gold, based on the conjugation efficiency of a typical IgG molecule. The exact amount may vary depending on the molecular weight, structure, and properties of the protein being conjugated.

MATERIALS PROVIDED:

- NG30-B009: Naked Gold® Sol 30 nm, 15 O.D./mL, 9 mL
- NG40-B009: Naked Gold® Sol 40 nm, 15 O.D./mL, 9 mL
- BUFA-001: Buffer Solution A - 1.0 mL
- BUFB-001: Buffer Solution B - 1.0 mL
- BUFC-001: Buffer Solution C - 1.0 mL
- BUFD-001: Buffer Solution D - 1.0 mL
- CDB-002: Conjugate Drying Buffer - 2.0 mL
- BLK-002: BSA Blocking Solution - 2.0 mL

MATERIALS REQUIRED BUT NOT PROVIDED:

- Glass (preferred) or PETG test tubes
- Pipettes and tips
- Vortex
- 1M Sodium Chloride (NaCl) Solution

PRECAUTIONS AND WARNINGS:

- Read all instructions before use.
- Wear disposable gloves when handling specimens and kit components.
- Good Laboratory Practices must be followed.
- Avoid cross contamination of reagents.
- Dispose in accordance with all local, regional, and national laws and regulations.
- Do not mix components of one kit lot with components from other lots.

L-PI-NGIB34-B018-02 Date Approved: 04/08/2026

- Do not use kit components beyond their expiration dates.
- Do not use reagents that show signs of contamination.

STORAGE AND STABILITY:

- Store the kit at 2-8°C.
- DO NOT FREEZE or expose to elevated temperatures.
- Keep all vials and bottles containing reagents and components sealed when not in use.
- Allow all reagents to reach room temperature, 20-25°C, before use.
- When stored as detailed above, the Gold-in-a-Box Conjugation Kit and individual components are stable up to the expiration dates printed on the labels.
- Discard any remaining components after their expiration dating.

SAMPLE PREPARATION:

Before starting a conjugation protocol, ensure that your ligand buffer is compatible with BioAssay Works' gold conjugation protocol. Avoid protein stabilizers (e.g. BSA), free amino acids (e.g. glycine), thiol compounds (e.g. DTT, Mercaptoethanol), EDTA, or Tris-based buffers and buffers that might interfere with the conjugation pH.

Ideally, the ligand must be at a concentration of 2 mg/mL or greater and should be in a phosphate buffer solution (1X PBS). Ligands at concentrations < 2 mg/mL should be in 0.5X PBS. If not, dialyze against 0.5X PBS.

The saturation point for 30 nm gold particles is approximately 45 µg/mL of 15 O.D./mL gold. Ideally, 12 µL of ligand at a concentration of 2 mg/mL is added to 0.5 mL of 15 O.D./mL gold. The saturation point for 40 nm gold particles is approximately 30 µg/mL of 15 O.D./mL gold. Ideally, 8 µL of ligand at a concentration of 2 mg/mL is added to 0.5 mL of 15 O.D./mL gold. These reactions may be modified or scaled as needed, depending on the concentration of the ligand.

CONJUGATION PROCEDURES:

Procedure for ligand with undetermined pI

1. Allow kit reagents and samples to warm to room temperature, 20-25°C.
2. Label ten (10) test tubes with the pH value (or tube #1 through tube #10) from Table 1.
3. Mix by inverting the bottle of Naked Gold Sol (NG30-B009 or NG40-B009) to re-suspend any settled particles. Quickly dispense 0.5 mL of Naked Gold Sol into the ten (10) labeled test tubes.
4. Use Table 1 to add varying amounts of Buffers A, B, C, and D to the appropriate test tubes and mix thoroughly with a low speed vortex.

Table 1. Various pH buffers

Tube	pH	Buffer A (BUFA-001)	Buffer B (BUFB-001)	Buffer C (BUFC-001)	Buffer D (BUFD-001)
1	5.4	9 µL	1 µL	-	-
2	6.6	8 µL	2 µL	-	-
3	7.3	6 µL	4 µL	-	-
4	7.8	4 µL	6 µL	-	-
5	8.2	2 µL	8 µL	-	-
6	8.4	-	-	10 µL	0 µL
7	8.8	-	-	8 µL	2 µL
8	9.2	-	-	6 µL	4 µL
9	9.6	-	-	4 µL	6 µL
10	10.1	-	-	2 µL	8 µL

5. Add 12 µL of the solution containing ligand at 2 mg/mL to each test tube when using NG30-B009. Add 8 µL of the solution containing ligand at 2 mg/mL to each tube when using NG40-B009. Mix thoroughly with a low speed vortex.
6. Allow the reaction to continue for a total of 30 minutes.
7. A deep purple or black color indicate that the ligand is below its isoelectric point, leading to cross-linking of individual gold sols and precipitation. These sols cannot be used and should be discarded. Only tubes with no change in color are recommended for immunological assays.
8. (Optional) Conjugated gold particles take on the properties of their ligands and become very stable in solutions of high ionic strength. An excellent way to test the effectiveness of the conjugation reaction is to combine 10 µL of coated gold sol (prior to the addition of the BSA Blocking Solution in Step 9) with 10 µL of 1M NaCl. Sols with incomplete coating will fall out of solution (turn black), while coated sols will remain stable (red). If the coating is not optimal, a 20% increase or decrease in ligand volume is often sufficient to yield an optimal coating procedure. A few cases require a 40% or more variation in ligand volume.
9. Stop the reaction by adding 50 µL of BSA Blocking Solution (BLK-002). Mix carefully and incubate for 5 minutes at room temperature. **NOTE:** In some conjugates that exhibit non-specific reactivity, it is often useful to allow the BSA Blocking Solution to react for up to an additional 16 hours to ensure complete coating of the conjugate.

The gold conjugate is now ready for use in a rapid assay at an optimal volume of 2 - 10 µL per test.