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Gold Nanoparticle Conjugation for Lateral Flow Assays: A Technical Guide

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A well-made gold conjugate is the single biggest factor in lateral flow sensitivity, background and lot-to-lot reproducibility. This guide gives new users a tested, five-step workflow for conjugating antibodies to Attogene colloidal gold by passive adsorption, from a 96-well pH screen to a working prototype strip.

1. Why the conjugate matters

The gold conjugate is the detection reagent in a lateral flow assay: it binds the analyte and produces the visible test line. Lateral flow tests now serve clinical, veterinary, agricultural, biodefense and environmental testing because they replace instruments, trained staff and a laboratory with a strip that gives an answer in minutes. That simplicity depends on a stable, active conjugate.

Component	Material	Function
Sample pad	Cellulose or glass fiber	Receives and conditions the sample
Conjugate pad	Glass fiber carrying the gold conjugate	Releases conjugate, which binds the analyte
Reaction membrane	Nitrocellulose	Holds the capture antibody (test line) and an anti-species antibody (control line)
Wick	Cellulose absorbent pad	Pulls sample through the strip by capillary action

The components sit on an adhesive backing card and run either as a dipstick or inside a cassette with a sample port and read window.

2. How antibodies bind gold

Antibodies adsorb passively to citrate-capped gold through three forces: ionic attraction to the negative citrate layer, hydrophobic contact with the gold surface, and dative bonds between gold and cysteine sulfur. No crosslinker is needed.

Two variables control the outcome:

- **pH.** Adsorption is most stable at or just above the antibody's isoelectric point (pI). Well below the pI, the positively charged protein bridges particles and aggregates them; well above it, the protein is repelled and binds poorly. Because pI varies by antibody, pH is found by screening (Step 1).
- **Amount of antibody.** Bare gold aggregates in salt, shifting from red to purple-gray. A complete protein coat prevents this. The salt challenge used in Steps 1 and 2 is therefore a direct readout of a stable coat.

Covalent coupling to carboxyl- or NHS-activated gold is an option for antibodies that lose activity on adsorption. Passive adsorption is simpler and works for most antibodies.

Attogene colloidal gold

Uniform particles give a predictable surface area, so the antibody loading found in a small screen holds at scale and from lot to lot. Attogene colloidal gold is manufactured in-house to these specifications:

Specification	Attogene colloidal gold	Benefit
Sizes	10, 30, 40, 60 nm (AU2012–AU2015)	40 nm is the standard starting point for lateral flow
Size distribution	CV below 12%	Consistent antibody requirement per particle
Shape	More than 95% spherical	Even protein coverage and a sharp absorbance peak
Batch-to-batch variation	±2 nm	Optimized conditions carry over to new gold lots
Membrane flow	Uniform	Reproducible line intensity from strip to strip

Pre-conjugated Protein A gold (AU2016) and streptavidin gold (AU2017) are also available for early feasibility work.

3. Before you start

Prepare all buffers in advance; adsorption is complete within seconds of mixing.

Reagent or equipment	Specification
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Reagent or equipment	Specification
Attogene colloidal gold	1 OD at λ_{max} ($\approx$ 520–530 nm), 40 nm typical; use as supplied
Antibody	Purified IgG, $\geq$ 1 mg/ml
MES buffer	22 mM, pH 5.5, 6.0, 6.5
HEPES buffer	22 mM, pH 7.0, 7.5
Borate buffer	22 mM, pH 8.0–10.0 in 0.5-unit steps
NaCl	1.5 M
BSA	10% (w/v), protease- and IgG-free
Tween 20	10% (v/v)
96-well plate	Clear, flat-bottom (Attogene CO2033)
Plate reader	520 nm and 580–600 nm; 400–700 nm scan preferred
Centrifuge	Up to $\sim$ 15,000 $\times$ g

Prepare the antibody first. BSA, gelatin, glycerol, Tris, glycine and sodium azide compete for the gold surface or destabilize it, and are the most common cause of failed conjugations. Buffer-exchange the antibody into 10 mM HEPES (or the chosen buffer) using Attogene 7K MWCO desalting columns (CO2027), then confirm concentration by A280.

4. Step 1: Screen pH

This screen finds the pH at which the antibody stabilizes the gold against salt.

1. Add 100 μ l of 1 OD colloidal gold to each well.
2. Add 10 μ l of 22 mM buffer, one pH per well, from pH 5.5 to 10.0 in 0.5-unit steps. Include a buffer-only well (no antibody) as the aggregation control.
3. Add 10 μ l of antibody at 100 μ g/ml in the matching buffer and mix immediately. This gives 10 μ g antibody per ml of gold.
4. Incubate 15 minutes at room temperature.
5. Add 25 μ l of 1.5 M NaCl ($\approx$ 0.26 M final) and mix.
6. Read after 5–10 minutes.

Reading the result. Stable wells stay ruby red; aggregated wells turn purple, then gray-blue. For an objective call, read A520 and A600: a stable colloid keeps a high A520 and a low A600/A520 ratio. Choose the pH in the middle of the passing range for the most margin.

Many monoclonal antibodies conjugate well at pH 7.5. HEPES is convenient here because it dissolves near pH 7.5 with little titration.

5. Step 2: Titrate the antibody

This screen finds the minimum amount of antibody that stabilizes the gold at the optimal pH.

1. Add 100 μ l of 1 OD colloidal gold to each well.
2. Add 10 μ l of 22 mM optimal buffer.
3. Add 10 μ l of antibody at each stock concentration below and mix immediately.
4. Incubate 15 minutes at room temperature.
5. Add 25 μ l of 1.5 M NaCl and mix.
6. Read after 5–10 minutes, as in Step 1.

Antibody stock (μ g/ml)	Antibody per well (μ g)	Loading (μ g per ml of gold)
100	1.0	10
75	0.75	7.5
50	0.50	5.0
37.5	0.375	3.75
25	0.25	2.5
18.75	0.1875	1.875
12.5	0.125	1.25
0 (control)	0	0

The lowest loading that keeps the well red is the minimum stabilizing amount. Carry the loading value into scale-up:

$$\text{Antibody } (\mu\text{g}) = \text{gold volume (ml)} \times \text{loading } (\mu\text{g/ml}) \times \text{gold OD} \times 1.1\text{--}1.2$$

The 10–20% excess allows for handling losses. The minimum stabilizing amount is not saturation; more antibody can raise signal, but any unbound antibody must be washed out (Step 3), or it will compete with the conjugate for analyte.

6. Step 3: Scale up, block and wash

Scale-up keeps the plate ratios unchanged; the example below makes 10 ml of conjugate from 1 OD gold with a minimum loading of 5 μ g/ml (60 μ g antibody with 20% excess).

1. Add 1 ml of 22 mM optimal buffer to 10 ml of gold in a low-protein-binding polypropylene tube.
2. Dilute 60 μ g of antibody to 1 ml in the same buffer.
3. Add the antibody to the gold in one smooth addition while vortexing gently. Never add gold to antibody; the local excess of particles causes partial aggregation.
4. Incubate 15–30 minutes at room temperature with gentle mixing.
5. Block: add 10% BSA to 1% final ($\approx$ 1.2 ml) and incubate 15–30 minutes.
6. Optional: spin at $\sim$ 500 $\times$ g for 5 minutes and discard any pellet of aggregates.

7. Pellet the conjugate (speeds below), discard the supernatant containing free antibody and BSA, and resuspend in conjugate diluent. Repeat once.

Particle size	Speed range	Time
20 nm	~6,000–15,000 × g	30 min
40 nm	~1,500–9,000 × g	20–30 min
60 nm	~700–4,000 × g	15–20 min

Start at the low end of the range: lower speeds give softer pellets that resuspend easily with less risk of aggregation, and we routinely pellet 40 nm conjugates at 1,500 × g. Lower speeds may need longer spins; adjust so the supernatant is nearly clear and the pellet stays soft. For larger batches, tangential flow filtration is an alternative; pre-wet the membrane with BSA solution to prevent conjugate loss.

7. Step 4: Store and check quality

Resuspend in a conjugate diluent such as 2–10 mM borate or Tris pH 8–8.5 with 1% BSA and 0.05–0.1% Tween 20. Add 5–10% sucrose or trehalose if the conjugate will be dried onto a pad, and a preservative (0.05% ProClin or sodium azide) for liquid storage. Adjust to a working concentration, typically 5–10 OD, and store at 2–8 °C, protected from light. **Never freeze a liquid gold conjugate**; ice crystals aggregate it irreversibly.

Release each lot against four checks:

Check	Pass criterion
UV-Vis spectrum, 400–700 nm	Peak red-shifted 2–5 nm vs. unconjugated gold; no shoulder above 600 nm
OD at λ_{max}	Matches the target working concentration
Salt challenge	Remains red after 1.5 M NaCl
Appearance	Clear ruby-red liquid; any pellet resuspends easily

Record pH, loading, blocking, gold lot and final OD for every lot.

8. Step 5: Test function on blank strips

A dot-spot test confirms that the conjugate binds analyte and identifies a working antibody pair before any membrane is striped. Attogene Blank 4.5 mm Lateral Flow Strips (AU2054) are pre-assembled for this: 4.5 × 60 mm strips with a glass-fiber conjugate pad, cellulose wick and high-protein-binding nitrocellulose, supplied in desiccant canisters with a 96-well plate.

1. Spot 0.5–1 μl of capture antibody at 0.1–2 mg/ml onto the nitrocellulose, stabilized with BSA or sucrose. Add a spot of anti-species antibody as the control.
2. Dry at 37 °C for 1–12 hours, or at 2–8 °C for 12–24 hours.
3. In a microwell, mix 80–200 μl of sample in running buffer (for example with 0.1% Tween 20 and 0.1–1% BSA) with 1–10 μl of 10 OD conjugate. Run a buffer-only negative alongside.

4. Insert the strip, conjugate pad down, and read after 5–30 minutes.
5. Pass: a strong test spot with analyte, none without, and a control spot in both.

Test every candidate antibody both as capture and as detector; pairs often work in only one orientation. To select membrane and pad materials at the same time, the Attogene Lateral Flow Development Starter Kit (AU2059) supplies 480 strips covering every combination of 4 nitrocellulose membranes, 2 wicks and 3 sample pads, plus 100 cassettes and 125 ml of buffer.

Screen gold lots, too. We recommend conjugating each antibody to several batches of gold and comparing them side by side on blank strips. Even within specification, an antibody can perform measurably better on one lot than another. Select the lot that gives the strongest specific signal with the lowest background, record its lot number with the antibody, and reserve enough of it for the project.

9. Troubleshooting

Most failures trace back to the antibody buffer, the pH or too little antibody; check these first.

Symptom	Likely cause	Fix
Gold turns purple when antibody is added	pH well below pI; antibody too concentrated; gold added to antibody	Raise pH; dilute antibody; always add antibody to gold
Every well aggregates in the salt challenge	Tris, glycine, azide, BSA or glycerol in the antibody	Desalt into 10 mM HEPES (CO2027 columns); recheck A280
Conjugate is stable but gives no signal	Epitope blocked or antibody denatured on adsorption; wrong pair orientation	Try another passing pH; swap capture and detector; consider covalent coupling
Weak test line	Low loading or conjugate OD; free antibody competing	Increase loading; raise OD; wash more thoroughly
High background or false positives	Incomplete blocking; aggregates trapped in membrane	Increase BSA; add a low-speed spin; add Tween 20 to running buffer
Pellet will not resuspend	Over-centrifuged	Reduce g-force or time; resuspend by pipetting
Lot-to-lot variation	Different gold size or OD; new antibody lot	Functionally screen several gold lots with each antibody and reserve the best; record OD and loading; re-titrate each antibody lot

10. Attogene products and services

AttoGene supplies every material in this workflow, and our development team can take on the work directly.

Product	Catalog no.	Use in this guide
Colloidal gold, 10 / 30 / 40 / 60 nm	AU2012 / AU2013 / AU2014 / AU2015	Conjugation (Steps 1–4)
Protein A gold; streptavidin gold	AU2016; AU2017	Ready-made conjugates for feasibility
Blank 4.5 mm Lateral Flow Strips	AU2054	Functional testing (Step 5)
Lateral Flow Development Starter Kit	AU2059	Membrane and pad selection
Lateral flow cassettes	CO2022	Prototype devices
Microspin desalting columns, 7K MWCO	CO2027	Antibody preparation
Protein Biotinylation Kit	CO2028	Biotinylated binders for streptavidin gold
RNase-Free Running Buffer Screening Pack	AU2035	Running buffer optimization
96-well plate for lateral flow	CO2033	Screens and microwell runs

Custom development. Our lateral flow services run from feasibility study to manufacturing scale-up and technical transfer, including antibody pair screening, colloidal gold conjugation, conjugate and sample pad optimization, buffer formulation, fluorescent reporters (quantum dots, europium, R-phycoerythrin), multiplex design, prototype manufacturing, validation, stability testing and reader integration.

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All products are for research and development use only.

Sources

- [AttoGene AU2054 Blank 4.5 mm Lateral Flow Strips data sheet](#)
- [AttoGene Lateral Flow Development Starter Kit \(AU2059\)](#)
- [AttoGene Custom Lateral Flow Assay Development](#)