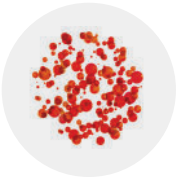
Gold

Protein Conjugation Protocol



Product	Product code	Quantity
Gold Colloid 20nm / 40nm / 60nm / 80nm	GCIKITDIAG/4	20ml
Gold Colloid 20nm / 40nm / 60nm / 80nm	GCIKITDIAG/7	100ml
Gold Colloid 5nm / 10nm / 20nm / 40nm	GCIKITLIFE/4	20ml

What is this protocol for?

Protein conjugation can be complex, especially when developing a reproducible, scalable product.

Conjugation of proteins to gold depends upon three separate but dependent phenomena:

- Ionic attraction between negatively charged gold and positively charged protein
- Hydrophobic attraction between the protein and the gold surface
- Dative binding between the gold conducting electrons and sulphur atoms which may occur within amino acids of the protein

This protocol shares a bit of our expertise to use with a gold sample kit and is suitable for assessment of potential conjugation conditions. The resultant conjugates provide a solid starting point for evaluation. However, further optimization may be needed before proceeding to full manufacturing scale.

Particle Selection

Each particle size has its advantages; however, the final selection is often closely linked to application, and the specific interactions with the particles, materials and components within the application.

BBI's Gold Colloid Starter Packs contain 20nm, 40nm, 60nm and 80nm particles at OD1 concentration, read at 520nm. These particle sizes are typically used in lateral flow applications. 40nm is most frequently used, based on its balance of visual signal and steric hinderance. However, 60nm and 80nm particles can give enhanced signal strength, whereas 20nm can give increased detection at lower levels- but these are all basedon the individual test format. It is often worthwhile assessing multiple particle sizes.

Gold Colloid

BBI Gold Colloids are prepared to a concentration of 0.01% Au and are ready for use. They must be adjusted to a suitable pH before use- this is specific to the protein being used.

Colloids can be adjusted within the range pH 5 to pH 9.5 without harming the colloid. Outside of these ranges or in the presence of excess salts the colloid may precipitate, it is advisable to test small volumes first.

Do not freeze an unconjugated gold colloid.

Get in touch with our gold conjugation experts: info@bbisolutions.com
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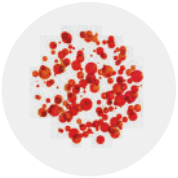

Required Components

BBI Gold Colloid Starter Pack
Protein for conjugation
2mM di-sodium tetraborate buffer
10% NaCl solution
HCl and NaOH solutions for pH adjustment
LoBind 1.5mL Eppendorf tubes

Protocol

This protocol can be repeated for each pH to be assessed. Adjust the pH of the 2mM di-sodium tetraborate buffer as required.

The titration series below will depend on individual protein. Volumes below are typical for antibodies but may need amending for smaller proteins if results are not as expected.

For 60nm and 80nm particle sizes, you may want to amend the volumes below to allow for more protein loadings between 0 and 1µg/mL.

For 20nm particle sizes, you may need to amend the volumes below to allow for higher protein loadings than 5µg/mL.

Step **Detail**

1. Adjust pH of 10mL Gold Colloid
2. Dilute protein to 0.05µg/mL with buffer
3. Adjust pH of diluted protein
4. In a series of LoBind Eppendorfs, add the following:

	Protein Loading (µg/mL)					
	0	1	2	3	4	5
Protein (µL)	0	20	40	60	80	100
Buffer (µL)	100	80	60	40	20	0
Gold Colloid (µL)	1000	1000	1000	1000	1000	1000

5. Mix and allow to incubate for ten minutes, then add 200µL of 10% NaCl solution to each Eppendorf
6. Wait a further ten minutes before recording the result
7.
   - A protected conjugate (fully coated with protein) will be red
   - An unprotected conjugate will appear purple
   - A severely under-protected conjugate will appear blue

   A red conjugate indicates suitable protein loading. If all Eppendorfs change purple in colour, the pH is unsuitable for the protein being assessed.

Protein Conjugation Protocol

Next Steps

You have successfully identified potential conjugation conditions.

Beyond this protocol, many other factors become important in being able to produce a stable, reproducible, large-scale conjugate built for manufacture.

BBI's Custom Conjugation service offers access to our scientists' years of experience and expertise to support your ongoing projects.

Why BBI?

Used in over 400 million assays every year, our gold manufacturing technique guarantees:

- Uniformity with ≤5% odd shapes and a CV of ≤8%, the uniform shape and size of BBI gold ensures even antibody binding, giving reliable results in your assay
- High stability – a minimum one year shelf life ensures a settled test manufacturing regime, saving you time and wastage
- Scalability – our batch sizes go up to 340L (40nm) to ensure you have continuous supply
- Quality – BBI gold must pass strict quality procedures before it's released to our customers, guaranteeing impressive performance characteristics at scale

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