Gold Nanoparticles Conjugation Kit-NHS
PRODUCT DATA SHEET

Gold Nanoparticles Conjugation Kit-NHS

Description

Abvigen Gold Nanoparticles Conjugation Kit-NHS have been optimized for high efficiency onestep conjugations of proteins and other primary aminecontaining ligands to gold nanoparticles with diameters in the size range of 5 nm ~ 100 nm. The kit contains ready-to-use pre-made mixtures. No activation or manipulation of the gold nanoparticles is required prior to conjugation, which often results in poor performing conjugates. Simply mix your protein with the pre-activated NHS ester gold nanoparticles supplied in the kit to generate your conjugate.

Kits are available in convenient 3 or 10 small-scale reactions formats allowing multiple to be conjugated simultaneously and ready for use in 2.5 h or less. These kits are ideal for screening and optimization purposes prior to scale-up production. Scale up can be performed with our NHS-activated Gold Nanoparticle Conjugation MIDI kit. The precisely engineered gold surface on our NHS ester-activated gold nanoparticles results in high conjugation efficiency and stable conjugates while minimizing non-specific binding in your assay. For quality control of antibody and antigen conjugation our Conjugation QC Lateral Flow Dipstick Kit can be used.

For custom sizes, formulations or bulk quantities please contact our customer service department.

Website: www.abvigen.com Phone: +1 929-202-3014 Email: info@abvigenus.com

Kit Components

NHS-Activated Gold Nanoparticles (lyophilized)

Protein Re-suspension Buffer

Reaction Buffer

Quencher Solution

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Characteristics

Gold surface: NHS-ester (spacer between gold surface and NHS-group)

Core diameter: Available with diameters from 5 nm ~ 100 nm

Optical density (OD): OD=20 when the contents of each vial is dissolved to a final volume of 100 ul (1 ml for MIDI Kit).

Supplied in ready to use lyophilized format.

Features & Benefits

Results in covalently bound ligand and more stable conjugate.

Fast and convenient one-step conjugation reaction with no pre-activation requirements.

Spacer between the gold nanoparticle surface and conjugated ligand minimizes effects on the tertiary protein structure, which can lead to poor performing conjugates, which is a common problem seen in conjugates prepared by passive adsorption.

Applications

Ideal for development of protein gold conjugates for use in applications such as blotting, lateral flow assays, microscopy and transmission electron microscopy (TEM).

Storage

All components of this kit should be stored at -20°C. If stored unopened and as specified, Abvigen NHS-activated gold nanoparticles are stable for at least 3 months.

Factors to Consider Prior to Conjugation

The protein/antibody or other ligand to be conjugated needs to be in a purified form, and proper care must be taken to ensure that the ligand stock is devoid of the following for proper functionality:

- No additional protein additives such as BSA
- Avoid free amino acids (e.g. glycine)
- Avoid common thiol additives such as DTT, TCEP and mercaptoethanol
- Avoid EDTA
- Avoid primary amine containing buffers or components (e.g. Tris)
- Avoid use of strong buffers that might change the pH of the conjugation reaction. See paragraph below for recommended buffers for optimal performance of the kit.

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If your protein/antibody stock contains any of the above, dialyse or use a desalting column to transfer your ligand into a compatible buffer such as sodium phosphate, MES, MOPS or HEPES. If contaminating proteins such as BSA is present, the protein needs to be purified prior to conjugation.

Conjugation Protocol

A recommended starting protocol for conjugation can be found below. Note that the amount of protein added may need to be optimized for your particular protein.

1. Allow all reagents to warm to room temperature before use.

2. Using the supplied protein re-suspension buffer, dilute or dissolve your protein/antibody to the final concentration suitable for the particular gold nanoparticle size to be conjugated as indicated in table II.

Note: For effective conjugation, the purity of the protein needs to be considered. Any other molecules containing primary amines (e.g. TRIS) or other contaminating proteins (e.g. BSA) may compete with the protein to be conjugated and hence severely reduce the conjugation efficiency and should therefore be avoided. Consider using BSA Removal Kit for Nanoparticle Conjugation.

3. In a microcentrifuge tube combine your diluted protein sample with reaction buffer according to the table below.

	3 or 10 Small Scale Reaction Format Kits	**Midi Kits**
Reaction Buffer	60 µl	600 µl
Diluted Protein Solution	48 µl	480 µl
Total Volume	108 µl	1080 µl

4. Transfer 90 µl (900 µl for the Midi Kit) of your protein/reaction buffer mix prepared in step 3 to one of the vials containing lyophilized NHS-activated gold nanoparticles and immediately mix well by pipetting up and down.

Note: Do not resuspend the lyophilized NHS-activated gold nanoparticles in buffer prior to addition of protein. NHS rapidly hydrolyzes in aqueous solution and may result in loss of conjugation efficiency.

5. Incubate the vial at room temperature for 2 h.

6. Add 10 µl (100 µl for MIDI Kit) of quencher solution to the vial and incubate for 5 min to stop the reaction.

7. (Optional Step) - Add 10 µl (100 µl for Midi Kit) of 10% bovine serum albumin (BSA) to the vial and incubate for 5 min to block.

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8. Using a microcentrifuge, centrifuge the vial for 30 min using the appropriate speed for the gold nanoparticle size you are using according to table I.

9. Discard the supernatant containing unbound protein.

10. Add 1 mL of gold conjugate storage buffer to the vial to re-suspend your conjugate.

11. Repeat step 8-9 to wash away additional unbound protein.

12. Add 100 µl (1 mL for Midi Kit) of gold conjugate storage buffer to the vial to re-suspend your conjugate. Brief sonication might be required to fully redisperse the conjugate.

* Note: A gold conjugate storage buffer is not supplied with the kit. Use a standard biological buffer compatible with your protein.

A recommended storage buffer for an antibody gold conjugate is 20 mM Tris (pH 8.0), 150 mM NaCl supplemented with 1% (w/v) BSA and 0.025% Tween 20.

13. Obtain a UV-VIS spectra of the conjugate using a spectrophotometer and dilute to an optical density of 10 using gold conjugate storage buffer.

14. Store your protein conjugate at 4°C until use.

15. (Optional Step) - Perform a conjugation quality control test to ensure a successful conjugation reaction using our Conjugation QC Lateral Flow Dipstick Kit.

Your conjugate is now ready for use!

Purification of Nanoparticle Conjugates Using Column

1. IMPORTANT: If your product or any downstream applications are sensitive to glycerine, make sure to rinse the filtration device with ddH_2O or buffer before use. Trace amounts of glycerine are present in the filtration membrane to prevent drying out.

2. Transfer your conjugated sample into the appropriate Column.

Note I. Ensure that the molecular weight cut-off (MWCO) of the Column is suitable for the components being filtered out (i.e., the reactants being removed should have a lower molecular weight than the cut-off of the column). The recommended MWCO is 100 kDa for nanoparticle products.

Note II. Do not overfill the Column, such that there is still some space left. This will mitigate any leakage between the two column components during centrifugation.

3. Using a suitable centrifuge, centrifuge the columns according to the table below, making sure to always use a counterbalance. If there is more volume than the filter device can hold, the remainder of the sample or any wash solutions can be poured into the unit on top of the purified product and

centrifuged again. Make sure to always empty contents collected at the bottom of the tube between each centrifugation.

Table 1. Recommended centrifugation speeds and times for different volume Column.

Column Size	Centrifugation Speed (x g)	Centrifugation Time
0.5 mL	10,000	10 min
4 mL	1,700	10 min
15 mL	1,700	10 min

Note. Centrifugation times will vary based on the MWCO, with smaller MWCO devices requiring longer centrifugation. If the remaining volume of purified product is more than desired, subsequent centrifugations can be done.

4. Following centrifugation, carefully collect the purified product using a micropipette. A small volume of collection buffer can be used to rinse and collect any leftover product on the membrane.

Note: The Column can be re-used but ensure that the membrane does not dry out between uses. In the event of drying out, the Column is no longer useable.

5. The purified product is now ready for analysis and any subsequent downstream applications.

Notes

This product is for R&D use only, not for drug, household, or other uses.

Table I. Recommended centrifugation force by gold nanoparticle size.

Gold Nanoparticle Diameter	Centrifugation Force
5 nm	100 kDa MWCO Spin Column
10 nm	17,000 x g, 1h or 100kDa MWCO Spin Column
15 nm	17,000 x g
20 nm	10,000 x g
30 nm	2,500 x g
40 nm	1,400 x g
50 nm	1,100 x g
60 nm	900 x g
70 nm	700 x g
80 nm	600 x g

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90 nm	500 x g
100 nm	400 x g

Table II. Suggested protein concentrations to be used for step 2 in the conjugation protocol above based on the gold nanoparticle size to be conjugated. Note that the concentrations in the table below are optimized for an antibody with a molecular weight of 150 kDa. For proteins differing significantly in molecular weights the amounts indicated might need to be optimized for optimal performance.

Gold Nanoparticle Diameter	**Suggested Protein Concentration**
5 nm	5 mg/ml
10 nm	3 mg/ml
15 nm	2 mg/ml
20 nm	1 mg/ml
30 nm	1 mg/ml
40 nm	0.5 mg/ml
50 nm	0.5 mg/ml
60 nm	0.5 mg/ml
70 nm	0.5 mg/ml
80 nm	0.5 mg/ml
90 nm	0.5 mg/ml
100 nm	0.5 mg/ml

Ordering Information

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