Gold Nanoparticles Conjugation Kit-Maleimide
PRODUCT DATA SHEET

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Description

Abvigen Gold Nanoparticles Conjugation Kit-Maleimide have been optimized for high efficiency one-step conjugation of thiol modified ligands such as oligonucleotides, antibodies, antibody fragments, proteins, and peptides, 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 maleimide gold nanoparticles supplied in the kit to generate your conjugate.

Kits are available in convenient 3 or 10 small-scale reaction formats allowing multiple to be conjugated simultaneously and ready for use in 1.5 h or less. These kits are ideal for screening and optimization purposes prior to scale-up production. Scale up can be performed with our Maleimide-activated Gold Nanoparticle Conjugation MIDI kit. The precisely engineered gold surface on our Maleimide-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

Maleimide Gold Nanoparticles (lyophilized)

Ligand Resuspension Buffer

Reaction Buffer

Quencher (lyophilized)

1378 US-206 Ste 6-126, Skillman, NJ USA
Email: info@abvigenus.com
Tel: 1-816-388- 0112 Fax: 1- 888-616-0161

Characteristics

Gold surface: Maleimide (spacer between gold surface and Maleimide 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 Vial).

Supplied in ready to use lyophilized format.

Features & Benefits

Versatile reagent

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

Oriented conjugation of antibody Fab' fragments

Covalently bound ligand and stable conjugate

Oriented biomolecules upon conjugation

Spacer between gold nanoparticle surface and conjugated ligand minimizes denaturation of biomolecules upon conjugates and enhances stability of conjugate.

Applications

Ideal for development of oligonucleotide or protein gold conjugates for applications such as blotting, lateral flow assays, microscopy, and transmission electron microscopy (TEM) studies, as well as drug and substrate delivery.

Storage

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

Conjugation Protocol

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

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

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

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Note:

(a) Maleimide reacts with thiol groups. Depending on the type of protein for conjugation, cleavage of disulfide bonds, or addition of sulfhydryl groups might be necessary prior to conjugation.

(b) For effective conjugation, avoid any other molecules containing thiol or contaminating proteins (e.g. BSA), which would compete with your ligand for binding sites. Consider using BSA Removal Kit for Nanoparticle Conjugation.

3. In a microcentrifuge tube, combine your diluted ligand from Step 2 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 ligand	48 µl	480 µl
Total Volume	108 µl	1080 µl

4. Transfer 90 µl (900 µl for the Midi Kit) of your ligand solution prepared in Step 3 to one of the vials containing lyophilized maleimide gold nanoparticles and immediately mix well by pipetting up and down.

5. Incubate the vial at room temperature for 1 h.

6. Add 10 µl (100 µl for MIDI Kit) of quencher solution* to the vial and incubate for 15 min to stop the reaction.

*The quencher is supplied in a lyophilized format and should be reconstituted with 100 µl of ddH_2O just prior to use. Any remaining quenching solution should be stored at -20°C.

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

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

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70 nm	400 x g
80 nm	400 x g
90 nm	300 x g
100 nm	300 x g

8. Discard the supernatant containing unbound ligand.

9. Add 100 µl (1 mL for Midi Kit) of gold conjugate storage buffer to the vial to re-suspend your conjugate.

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

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

A recommended storage buffer for an oligonucleotide gold conjugate is 10 mM Sodium Phosphate (pH 7.0), 100 mM NaCl.

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

11. Store your gold conjugate at 4°C until use.

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.

Note

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

Table I. Suggested ligand concentrations for Step 2 in the conjugation protocol based on the gold nanoparticle size.

Gold Nanoparticle Diameter	Suggested Protein Concentration, mg/ml	Suggested Oligonuclotide concentration, µM
5 nm	5	250
10 nm	3	150
15 nm	2	100
20 nm	2	100
30 nm	1	50
40 nm	0.5	30
50 nm	0.5	30
60 nm	0.5	20

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80 nm	0.3	20
90 nm	0.3	10
100 nm	0.3	10

Ordering Information

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