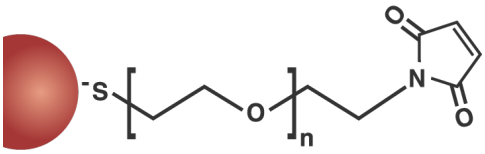
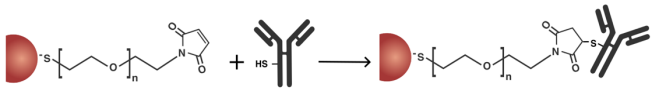
CYTODIAGNOSTICS

PRODUCT DATA SHEET

Maleimide Gold Nanoparticle Conjugation Kit



Maleimide



Description

Cytodiagnostics Maleimide Gold Nanoparticle Conjugation Kits 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 5nm-100nm.

The kit contains ready-to-use pre-made mixtures. No manipulation of the gold nanoparticles is required prior to conjugation. Simply mix your thiol-bearing biomolecule with the maleimide gold nanoparticles supplied in the kit.

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 hours or less. These kits are ideal for screening and optimization purposes prior to scale-up production. Scale up can be performed with Maleimide Gold Nanoparticle Conjugation MIDI kits.

Features & Benefits

- Versatile reagent
- Fast and convenient one-step conjugation reaction
- 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.

Kit Components

- Maleimide Gold Nanoparticles (lyophilized)
- Ligand Resuspension Buffer
- Reaction Buffer
- Quencher (lyophilized)

Maleimide Gold Nanoparticle Specifications

Gold surface: Maleimide (spacer between gold surface and Maleimide group)
Core diameter: Available with diameters from 5nm-100nm
Optical density (OD): OD=20 when the contents of each vial is dissolved to a final volume of 100ul. (1 mL for MIDI Vial)
Particles per ml: Core size dependant, please see table II.
Lambda max: Core size dependant, please see table II.
Supplied in ready to use lyophilized format.

Storage

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

Product Safety and Handling

This product is for R&D use only, not for drug, household, or other uses. Please review the material safety datasheet (MSDS) available online for proper safety and handling procedures.

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. With the supplied 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.

Canada, Europe, Asia, Pacific and Africa
919 Fraser Drive, Unit 11, Burlington, ON Canada L7L 4X8
Tel: 866-344-3954 Fax: 289-204-9100
www.cytodiagnostics.com

United States, Mexico, South and Central America
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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 (SR-08-01).

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	60µl	600µl
Diluted ligand	48µl	480µl
Total Volume	108µl	1080µl

4. Transfer 90µl (900µl 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 hour.

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

   **The quencher is supplied in a lyophilized format and should be reconstituted with 100 ul 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 minutes using the appropriate speed for the gold nanoparticle size you are using according to the table below.

Gold Nanoparticle Diameter	**Centrifugation Force**
5nm	*100kDa MWC Spin Column*
10nm	*17,000 x g, 1hr or 100kDa MWCO Spin Column*
15nm	*15,000 x g*
20nm	*5,500 x g*
30nm	*2,000 x g*
40nm	*900 x g*
50nm	*600 x g*
60nm	*500 x g*
70nm	*400 x g*
80nm	*400 x g*
90nm	*300 x g*
100nm	*300 x g*

8. Discard the supernatant containing unbound ligand.

9. Add 100ul (1ml 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 20mM Tris (pH 8.0), 150mM NaCl supplemented with 1% (w/v) BSA and 0.025% Tween 20.

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

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Purification of Nanoparticle Conjugates Using CytoColumn™

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 CytoColumn™ (see Page 5).

   Note I. Ensure that the molecular weight cut-off (MWCO) of the CytoColumn™ 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 CytoColumn™, 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 CytoColumn™.*

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

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

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	5	250
10	3	150
15	2	100
20	2	100
30	1	50
40	0.5	30
50	0.5	30
60	0.5	20
70	0.5	20
80	0.3	20
90	0.3	10
100	0.3	10

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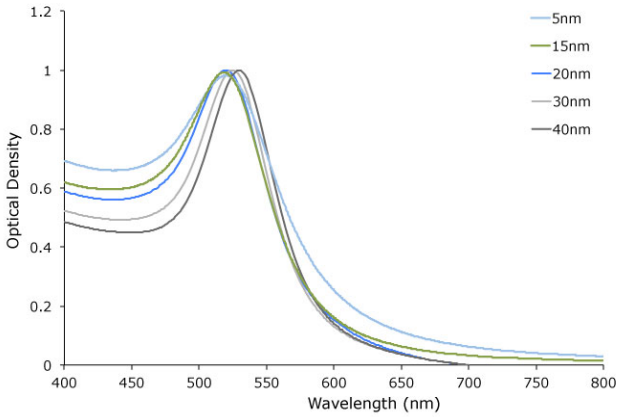
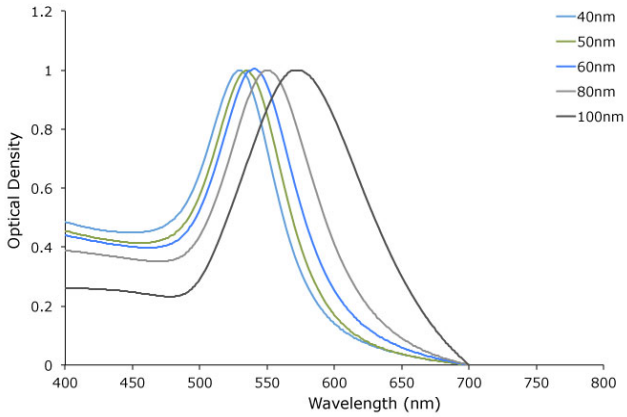
Table II. *Gold nanoparticle specifications by size. Please note that all values below are indicated at an optical density of 1 (OD/cm^{-1}) at their respective lambda max. At other optical densities the values needs to be adjusted (e.g. NPS/ml (@OD2) = 2 x NPS/ml (@OD1)).*

Diameter (nm)	Peak SPR Wavelength (nm)	NPS/ml	Wt. Conc. (mg/ml)	Molar Ext ($M^{-1}cm^{-1}$)	Size Dispersity (+/-nm)	Particle Volume (nm^3)	Surface Area (nm^2)	Surface/ Volume Ratio	Particle Mass (g)	Molar Mass (g/mol)	Molar Conc.
5	515-520	5.47E+13	6.94E-02	1.10E+07	<15%	6.54E+01	7.85E+01	1.2	1.27E-18	7.64E+05	9.08E-08
10	515-520	5.98E+12	6.07E-02	1.01E+08	<15%	5.24E+02	3.14E+02	0.6	1.02E-17	6.11E+06	9.93E-09
15	520	1.64E+12	5.61E-02	3.67E+08	<12%	1.77E+03	7.07E+02	0.4	3.43E-17	2.06E+07	2.72E-09
20	524	6.54E+11	5.31E-02	9.21E+08	<12%	4.19E+03	1.26E+03	0.3	8.12E-17	4.89E+07	1.09E-09
30	526	1.79E+11	4.91E-02	3.36E+09	<12%	1.41E+04	2.83E+03	0.2	2.74E-16	1.65E+08	2.98E-10
40	530	7.15E+10	4.65E-02	8.42E+09	<12%	3.35E+04	5.03E+03	0.15	6.50E-16	3.91E+08	1.19E-10
50	535	3.51E+10	4.45E-02	1.72E+10	<10%	6.54E+04	7.85E+03	0.12	1.27E-15	7.64E+08	5.83E-11
60	540	1.96E+10	4.30E-02	3.07E+10	<10%	1.13E+05	1.13E+04	0.1	2.19E-15	1.32E+09	3.25E-11
70	548	1.20E+10	4.17E-02	5.03E+10	<10%	1.80E+05	1.54E+04	0.086	3.48E-15	2.10E+09	1.99E-11
80	553	7.82E+09	4.06E-02	7.70E+10	<10%	2.68E+05	2.01E+04	0.075	5.20E-15	3.13E+09	1.30E-11
90	564	5.37E+09	3.97E-02	1.12E+11	<8%	3.82E+05	2.54E+04	0.067	7.40E-15	4.46E+09	8.92E-12
100	572	3.84E+09	3.89E-02	1.57E+11	<8%	5.24E+05	3.14E+04	0.06	1.02E-14	6.11E+09	6.37E-12





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Catalog Number	Description	Sizes
MG5K-5-X*	5nm Maleimide Gold Nanoparticle Conjugation Kit	3 reactions, 10 reactions, MIDI Kit
MG5K-10-X*	10nm Maleimide Gold Nanoparticle Conjugation Kit	3 reactions, 10 reactions, MIDI Kit
MG5K-15-X*	15nm Maleimide Gold Nanoparticle Conjugation Kit	3 reactions, 10 reactions, MIDI Kit
MG5K-20-X*	20nm Maleimide Gold Nanoparticle Conjugation Kit	3 reactions, 10 reactions, MIDI Kit
MG5K-30-X*	30nm Maleimide Gold Nanoparticle Conjugation Kit	3 reactions, 10 reactions, MIDI Kit
MG5K-40-X*	40nm Maleimide Gold Nanoparticle Conjugation Kit	3 reactions, 10 reactions, MIDI Kit
MG10K-50-X*	50nm Maleimide Gold Nanoparticle Conjugation Kit	3 reactions, 10 reactions, MIDI Kit
MG10K-60-X*	60nm Maleimide Gold Nanoparticle Conjugation Kit	3 reactions, 10 reactions, MIDI Kit
MG10K-70-X*	70nm Maleimide Gold Nanoparticle Conjugation Kit	3 reactions, 10 reactions, MIDI Kit
MG10K-80-X*	80nm Maleimide Gold Nanoparticle Conjugation Kit	3 reactions, 10 reactions, MIDI Kit
MG10K-90-X*	90nm Maleimide Gold Nanoparticle Conjugation Kit	3 reactions, 10 reactions, MIDI Kit
MG10K-100-X*	100nm Maleimide Gold Nanoparticle Conjugation Kit	3 reactions, 10 reactions, MIDI Kit

*X Indicates quantity, i.e. -1 for a 3-reaction kit, -2 for a 10-reaction kit and -3 for a MIDI kit.
For custom sizes and information on bulk quantities and prices please contact our customer service department.

Catalog Number	Description	Sizes
MWC-3-X*-Y*	MWCO Ultrafiltration Spin Columns, 3 kDa	0.5ml, 4ml, 15ml
MWC-10-X*-Y*	MWCO Ultrafiltration Spin Columns, 10 kDa	0.5ml, 4ml, 15ml
MWC-30-X*-Y*	MWCO Ultrafiltration Spin Columns, 30 kDa	0.5ml, 4ml, 15ml
MWC-100-X*-Y*	MWCO Ultrafiltration Spin Columns, 100 kDa	0.5ml, 4ml, 15ml

X* Indicates column volume, 05 for 0.5ml, 4 for 4ml, 15 for 15ml

Y* Indicates number of columns, 1 for 1 column, 5 for 5 columns. i.e. MWC-3-05-1, 3kDa cut-off, 0.5ml column pkg size of 1

Ordering Information

For ordering call 866-344-3954 or visit us online.

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