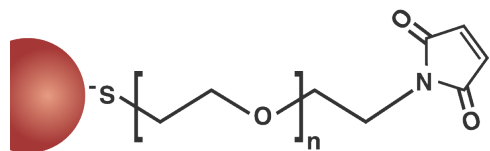


PRODUCT DATA SHEET

Maleimide Gold Nanoparticle Conjugation Kit



Maleimide



Description

Cytodiagnosics 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, Cytodiagnosics 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 (page 3).

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.

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 ddH₂O 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.

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).

| 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.

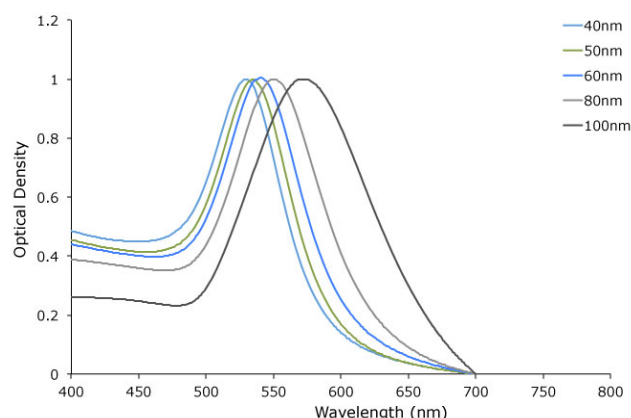
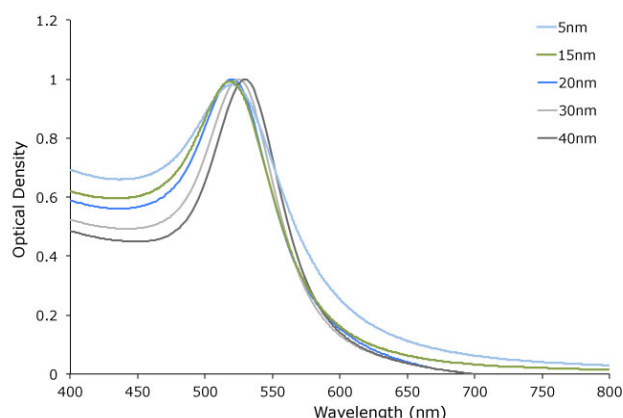
Your conjugate is now ready for use

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 Oligonucleotide 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 |

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 (@ $\text{OD}2$) = $2 \times \text{NPS}/\text{ml}$ (@ $\text{OD}1$)).

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



| Catalogue 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.

Ordering Information

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