

# InnovaCoat® GOLD Maleimide (20 OD) Conjugation Kit

Applicable to product codes 270 and 271

Release 6

01/03/2016

## Introduction

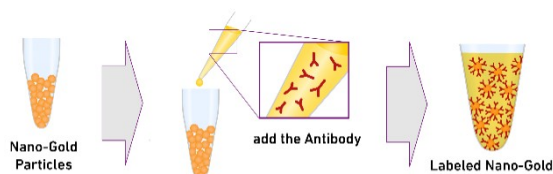
The InnovaCoat® GOLD Maleimide Conjugation Kit allows thiol groups (-SH) to be covalently attached to ultra-stable\* InnovaCoat® GOLD nanoparticles at very high OD quickly and easily (Figure 1). The hands-on time for the InnovaCoat® GOLD conjugation procedure is about 5 minutes, and the conjugate is ready to use within 1 hour.

*\* Maleimide groups are attached to the InnovaCoat surface coat, which does not detach from gold surfaces even under the most extreme conditions.*

The conjugation reaction is initiated simply by adding a solution of the thiol-activated molecule (e.g. thiol-activated biomolecules such as proteins, peptides, antibodies, antibody fragments or oligonucleotides) to the InnovaCoat® GOLD Maleimide nanoparticles.

Note that disulfide bonds in protein structures (e.g. between cysteines) must be reduced to thiol groups for them to react with maleimide reagents. Alternatively, thiol groups must be introduced into molecules that have no indigenous thiol groups. Free thiols (e.g.  $\beta$ -mercaptoethanol, DTT) must be excluded from samples and maleimide reaction buffers by desalting, because they will compete for coupling sites on the gold nanoparticles.

Single stranded oligonucleotides must be between 10 and 120 bases in length and contain a terminal thiol group, which must be added during synthesis. All commercial oligo suppliers offer this modification. The efficiency of conjugation is slightly higher with 5' aminated oligos.



**Figure 1.** The resulting covalent conjugates are far more stable than those prepared by adsorption of thiols to bare metal surfaces.

## Storage and Shipping

The InnovaCoat® GOLD Maleimide Conjugation Kit is shipped at ambient temperature in a tamper-evident polypropylene container.

The kit should be stored at  $-20^{\circ}\text{C}$  upon receipt and, once opened, unused vials should be stored with silica gel (the vials are moisture sensitive).

## Kit contents

1 vial (1 reaction Midi kit) or 3 vials (3 reaction Mini Kit) of InnovaCoat® GOLD Maleimide

1 vial of InnovaCoat® GOLD Maleimide Reaction Buffer

1 vial or bottle of InnovaCoat® GOLD Maleimide Diluent

1 vial of InnovaCoat® GOLD Maleimide Quencher

## Considerations prior to labeling

- The biomolecule to be conjugated must be purified and thiolated.
- Oligonucleotides should be HPLC purified; this is not necessary for proteins or antibodies.
- Free thiols in the biomolecule buffer (e.g.  $\beta$ -mercaptoethanol, DTT) must be avoided: a desalting step post reduction is mandatory if thiols are used to reduce the disulphide bonds.

The kit is compatible with PBS, MOPS, HEPES, sugars, salts and detergents.

## Amount of biomolecule

The optimum amount of biomolecule (which will influence the number of molecules per particle) may be application-dependent and must be determined by experimentation; you may need to conjugate different amounts of biomolecule to optimize your assay.

For example, around 2.5  $\mu\text{g}$  and 5  $\mu\text{g}$  of thiol-activated IgG antibody for 40 nm and 20 nm Mini vials respectively are likely to give optimal results. The suggested antibody amounts for a Midi vial are exactly 10 times the ones optimized for the Mini vial (e.g. 25  $\mu\text{g}$  and 50  $\mu\text{g}$  for 40 nm and 20 nm respectively). The recommended starting concentration for oligonucleotides is  $>100 \mu\text{M}$ , as this allows potentially interfering substances in the oligonucleotide preparation to be diluted out. If you are in any doubt regarding the suitability of the buffers/additives in which your biomolecule is supplied, please contact our Technical Support Team.

The recommended volume in which the biomolecule is added should be 45  $\mu\text{l}$  (Mini kit) and 450  $\mu\text{l}$  (Midi kit).

## Labeling protocol

- Allow all of the reagents to warm to room temperature.

(ii) Dilute the biomolecule in the InnovaCoat® GOLD Maleimide Diluent to produce a final volume of 40 µl (Mini kit) or 400 µl (Midi kit). Add 5µl or 50 µl of InnovaCoat® GOLD Maleimide Reaction Buffer respectively. Mix gently.

(iii) Remove the cap from the vial of InnovaCoat® GOLD Maleimide, mix and pipette the sample (biomolecule in InnovaCoat® GOLD Maleimide Reaction Buffer) directly onto the lyophilized material. Resuspend *gently* by withdrawing and re-dispensing the liquid once or twice using a pipette.

(iv) Place the cap back on the vial and leave it standing at room temperature (20-25°C); 30 minutes incubation is recommended for proteins or antibodies, while 60 minutes is recommended for oligonucleotides; longer incubation times have no negative effect on the conjugate.

(v) After incubating for 30 minutes (or more), add 5 µl (Mini kit) or 50 µl (Midi kit) of InnovaCoat® GOLD Maleimide Quencher (this is supplied as a lyophilized product and should be reconstituted in 100 µl of water; store surplus InnovaCoat® GOLD Maleimide Quencher at -20°C after use). The conjugate can be used after 20 minutes. You now have 50 µl (Mini kit) or 500 µl (Midi kit) of 20 OD conjugate.

Note: If you wish to exchange the InnovaCoat® GOLD conjugate into a specific buffer for your assay, centrifuge the conjugate in a microfuge at 9,000 g for 6 minutes for 40 nm gold nanoparticles or 20 minutes for 20 nm. Carefully remove the supernatant and add your preferred buffer. The buffer should not contain thiols; all other common lab materials are acceptable after the conjugate has formed.

#### Storage of labeled biomolecules

Storage at +4°C is recommended. A preservative may be desirable for long-term storage. Other storage conditions may also be satisfactory. The best conditions for any particular conjugate must be determined by experimentation.

For further information about Innova's products see our website: <https://www.innovabiosciences.com>

#### Technical Support Team

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