



## InnovaCoat® GOLD-Carboxyl 40 OD 40nm

Applicable to:

3400-0001 40nm 1ml pack

3400-0005 40nm 5ml pack

Release 1

22/07/2016

### Introduction

Carboxyl surface modification of gold nanoparticles is one of the most common functionalizations employed to covalently bind primary amine containing biomolecules (e.g. antibodies) using the water soluble carbodiimide N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC).

EDC is often used with NHS or sulfo-NHS to activate the carboxyl groups and then stabilize the intermediate ester formed which reacts with primary amines to form a stable amide bond.

InnovaCoat® GOLD-Carboxyl nanoparticles are optimized for single step EDC covalent coupling, without aggregation and crosslinking. Therefore, any EDC/NHS pre-activation and washing steps are unnecessary.

Another advantage over the traditional EDC/NHS coupling is the speed of the labeling process: the conjugate is ready to use in less than 35 minutes.

### Shipping and storage

InnovaCoat® GOLD-Carboxyl 40 OD 40nm bottles are shipped at ambient temperature in a small tamper-evident polypropylene container. On receipt, store the product at +4°C.

The shelf life is 36 months.

### Buffer composition

InnovaCoat® GOLD-Carboxyl 40 OD 40nm nanoparticles are shipped in 10mM EPPS pH 8.5. If you wish to exchange the InnovaCoat® GOLD-Carboxyl 40nm into a different buffer, centrifuge the particles in a microfuge at 2,500g for 10 minutes. Carefully remove the supernatant, gently tap the pellet and add your preferred buffer.

It is important to avoid substances that have a very high affinity for gold (e.g. thiols).

### Content and required materials

Content:

3400-0001 1ml bottle            or  
3400-0005 5ml bottle

Required materials:

- EDC (Sigma, prod code: 03449, Molecular Weight: 191.70)

- MES (Sigma, prod code: M2933)
- Antibody or protein to be conjugated (ideally > 1mg/ml in a 10mM buffer)
- 1X TBS, 0.05% Tween20
- BSA

### Antibody buffer considerations

There are a number of common buffer components that have a substantial negative effect on the conjugation efficiency. This decreases the amount of antibody/protein that will be coupled to each nanoparticle. To prevent this we advise conjugating only from stock antibodies that are at least 1mg/ml, which allows some dilution of interfering substances, or that are already provided in a suitable buffer.

Please see the table below for recommended buffer conditions and components of the antibody to be labeled.

If your antibody buffer is not compatible with our kits, our AbPure antibody purification kit range allows you to quickly and simply purify your antibody and is fully compatible with InnovaCoat® GOLD-Carboxyl. Please consult the kit protocols to see the antibody amount/volume suitable for the conjugation reaction.

|                                                                                                                   |         |
|-------------------------------------------------------------------------------------------------------------------|---------|
| pH                                                                                                                | 6.0-8.5 |
| Amine-free buffer* (e.g. MES, MOPS, HEPES)                                                                        | ✓       |
| Sugars                                                                                                            | ✓       |
| PBS*                                                                                                              | ✗       |
| Sodium Azide                                                                                                      | ✗       |
| BSA                                                                                                               | ✗       |
| Gelatin                                                                                                           | ✗       |
| Tris                                                                                                              | ✗       |
| Glycine                                                                                                           | ✗       |
| Carboxylic acids (e.g. EDTA, citrate)                                                                             | ✗       |
| Nucleophilic components (primary amines, e.g. amino acids or ethanolamine and thiols e.g. mercaptoethanol or DTT) | ✗       |

\* Note that relatively weak buffers (e.g. 10mM) are strongly preferred so that the pH conditions of the covalent reaction are not significantly altered upon addition of the antibody. Antibody concentrations >10mg/ml in PBS are acceptable.

### Antibody/protein conjugation suggested method

1. Prepare 100mM and 10mM MES buffers at pH 5.0.
2. Dilute the antibody or protein to be conjugated down to 0.05-0.5mg/ml in 10mM MES pH 5.0. You may need to conjugate different amounts of antibody/protein to optimize your assay. For IgGs we recommend testing

0.1mg/ml in the first instance, although slightly lower or higher concentrations can be explored to optimize performance in your particular application.

3. Prepare a fresh solution of 50mM EDC by adding 520ul of ultrapure water to 5mg of EDC and mix well.
4. Dilute the EDC down to 1mM by mixing 20ul 50mM EDC stock solution with 980ul water.

The EDC solution is not stable, use immediately

5. Add to 50ul 40nm InnovaCoat® GOLD-Carboxyl 40 OD:
  - 10ul 100mM MES pH 5.0 (step 1)
  - 20ul of diluted protein/antibody (step 2)
6. Add 20ul 1mM EDC to the gold/protein and mix well (step 3 and 4).
7. Incubate for 20 minutes at room temperature with mixing.
8. After the incubation, add 1ml of 1XTBS pH 8.0, 0.05% Tween20.
9. Spin the gold conjugate down by centrifugation for 10 minutes at 2,500g.
10. Carefully remove the supernatant without dislodging the pellet.
11. Tap the pellet and gently add 90ul of 1XTBS pH 8.0, 0.05% Tween20, 0.5% BSA to obtain around 20 OD of conjugate. Mix well. For long term storage, avoid BSA in your buffer.

### Conjugate concentration determination

The maximum absorbance for the 40nm IC-GOLD is at 530nm. To determine the effective concentration of the conjugate obtained we advise to measure the absorbance of light at 530nm using an UV-vis spectrophotometer after diluting your sample (e.g. if the conjugate is at 20 OD and is diluted 1:20 the  $Abs_{530nm}$  for a 1 cm light path is expected to be around 1 AU).

### Storage of conjugate

For any new conjugate, initial storage at 4°C is recommended. Do not store the conjugate at -20°C.

The bond between the nanoparticle and antibody is covalent, via the InnovaCoat surface, which means that the conjugates are very stable. Stability of individual antibodies on storage may vary and will need to be checked experimentally.

For additional applications please contact our Technical Support: [technical.enquiries@innovabiosciences.com](mailto:technical.enquiries@innovabiosciences.com)

### Related products and services

InnovaCoat® GOLD 20 OD 40nm Gold Conjugation kit (230-0005; 230-0010; 230-0015)

InnovaCoat® GOLD-Maleimide 20 OD 40nm Gold Conjugation kit (270-0005; 270-0015)

InnovaCoat® GOLD-Hydrazide 10 OD 40nm Gold Conjugation kit (280-0000; 280-0100; 280-0300)

InnovaCoat® GOLD Custom Services, including supply of bulk material, optimization of covalent antibody conjugates, covalent attachment of small molecules and ligands.

Visit our website: [www.innovabiosciences.com](http://www.innovabiosciences.com) or email [info@innovabiosciences.com](mailto:info@innovabiosciences.com) for more information.

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