

InnovaCoat® GOLD (20 OD) Conjugation Kit

Applicable to: 228-0005 3 Reaction Mini Kit
229-0010 10 Reaction Mini Kit
230-0015 1 Reaction Midi Kit
231-0015 1 Reaction Midi Kit

228-0010 10 Reaction Mini Kit
229-0015 1 Reaction Midi Kit
230-0050 1 Reaction Maxi Kit
232-0005 3 Reaction Mini Kit

228-0015 1 Reaction Midi Kit
230-0005 3 Reaction Mini Kit
231-0005 3 Reaction Mini Kit
232-0010 10 Reaction Mini Kit

229-0005 3 Reaction Mini Kit
230-0010 10 Reaction Mini Kit
231-0010 10 Reaction Mini Kit
232-0015 1 Reaction Midi Kit

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INTRODUCTION

The InnovaCoat® GOLD conjugation kit allows antibodies or proteins to be covalently attached to ultra-stable InnovaCoat GOLD nanoparticles at very high OD quickly and easily. InnovaCoat GOLD has a protective surface coat that can withstand the most extreme conditions (e.g. 2.5M NaOH at 70°C for >1 hour).

InnovaCoat GOLD nanoparticles in this kit are freeze dried. The conjugation reaction is initiated simply by reconstituting the dry mixture with your antibody, which becomes attached (via lysine residues) to the InnovaCoat surface.

The hands-on time for the InnovaCoat GOLD conjugation procedure is about 2 minutes and the conjugate is ready to use within 20 minutes. The researcher simply pipettes the biomolecule into a vial containing the gold nanoparticles (Figure 1).

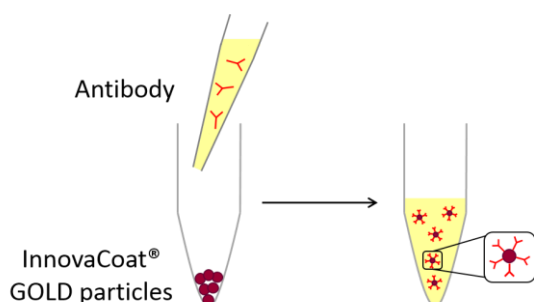


Figure 1. InnovaCoat® GOLD conjugation

The resulting covalent conjugates are more stable than those prepared by passive adsorption methods. Moreover, unlike passive methods, the coating procedure is not dependent on the isoelectric point of the antibody, thus extensive trials at different pH values are not required; all antibodies react at a single fixed pH.

KIT CONTENTS

- 1 glass vial (Midi* kit) OR 3 or 10 cryotubes (Mini* Kit) of InnovaCoat® GOLD (dependent on kit)
- 1 vial or bottle of reaction buffer
- 1 vial or bottle of antibody diluent
- 1 vial of quencher

*Each Midi vial gives sufficient conjugates for ~500 lateral flow tests

*Each Mini vial gives sufficient conjugates for ~50 lateral flow tests

SHIPPING AND STORAGE CONDITIONS

The kit is shipped at ambient temperature in a tamper-evident polypropylene container.

Store the kits at -20° C upon receipt.

The quencher, reaction buffer and antibody diluent can be stored at either 4°C or -20°C.

INSTRUCTIONS

1. Allow all of the reagents to warm to room temperature.
2. Dilute your stock antibody with the antibody diluent provided in the kit to recommended concentrations as below:

	10nm/20nm	40nm/60nm	80nm
Diluted antibody stock	0.25mg/ml	0.1mg/ml	0.05mg/ml

Note: If you wish to examine the effect of varying the amount of antibody, make additional stocks but do not change the volume of antibody added (see table below).

In order to vary the amount of antibody added, you must change the concentration of the stock antibody and use a fixed volume.

3. In a microfuge tube add the reaction buffer and your now diluted antibody according to the table below and mix gently:

	Mini kit	Midi kit
Reaction buffer	42µl	420µl
Diluted antibody	12µl	120µl

Note: For either type of kit you will have more mixture than you will actually use in the conjugation reaction.

4. EITHER add 45µl of reaction buffer/antibody mixture to a Mini vial OR add 450µl to a Midi vial, and reconstitute the InnovaCoat GOLD by gently pipetting up and down. Leave the reaction for 15 minutes at room temperature.
5. After 15 minutes, add the quencher to stop the reaction, and mix well, but gently. You must use the correct amount of quencher for your size of vial:

	Mini kit	Midi kit
Volume of Quencher	5µl	50µl

6. Leave the reaction for 5 minutes. You now have 20 OD conjugate (50µl for Mini kits and 500µl for Midi kits). Dilute further as required for your application.

Note: For a conjugate 100% free from unbound antibody we recommend to wash the particles adding 10 times the volume of the quencher diluted 1:10 in water to the conjugate (i.e. 1ml 1:10 diluted quencher to 100µl of conjugate) and then centrifuge it in a microfuge at:

- 9,000g 10 minutes (40,60 and 80nm gold conjugates)
- 9,000g 20 minutes (20nm gold conjugates)
- 21,500g 45 minutes (10nm gold conjugates)

Carefully remove the supernatant, gently tap the pellet and add the quencher diluted 1:10 in water for long term storage in the fridge (up to 1 year) or 1:10 diluted quencher with addition of 0.5 - 2% BSA for LFA or your preferred buffer. It is important to avoid substances that have a very high affinity for gold (e.g. thiols).

AMOUNT AND VOLUME OF ANTIBODY

The optimum amount of antibody (which will influence the number of antibody molecules per particle) depends on the size of the nanoparticles (surface area) and on the application; hence you may need to conjugate different amounts of antibody to optimize your assay. The table below shows the recommended initial amounts of antibody. However slightly lower or higher concentrations can be explored to optimize performance in your particular application. Moreover, for some antibodies, diluting 1:5 the reaction buffer in water prior to conjugation may be beneficial to your conjugation reaction.

Pack size	10nm/20 nm	40nm/60nm	80nm
Mini kit	2.5ug	1 µg	0.5 µg
Midi kit	25ug	10 µg	5 µg

BUFFER CONSIDERATIONS

Please see the below table for recommended buffer conditions and components:

Buffer Components	
pH	6.5-8.5
Amine free buffer * (e.g. MES, MOPS, HEPES)	✓
Sugars	✓
PBS*	✗
Thiomersal	✗
Thimerosal	✗
Merthiolate	✗
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.

MY BUFFER DOESN'T FIT THE REQUIREMENTS

If your antibody buffer is not compatible with our kits, we have developed the [AbPure antibody purification kit](#) range that allows you to quickly and simply purify your antibody and is fully compatible with InnovaCoat® GOLD.

Please consult the kit protocols to see the antibody amount/volume suitable for each kit.

STORAGE OF CONJUGATES

For any new conjugate, initial storage at 4° C is recommended. The quencher added at the end is a good conjugate storage buffer. **Do not store the conjugate at -20°C.** The bond between the nanoparticle and antibody is covalent, which means that the conjugates are very stable.

The determining factor for conjugate stability will be the antibody itself, as it will be first to degrade. Therefore as long as your antibody is stable, the conjugate will be stable as well.

MEASURING CONJUGATE CONCENTRATION

The maximum absorbance (Abs_{max}) for IC-GOLD is size dependent:

10nm	20nm	40nm	60nm	80nm
520nm	528nm	530nm	540nm	550nm

To determine the effective concentration of the conjugate obtained we advise to measure the Abs_{max} using an UV-vis spectrophotometer after diluting your sample to an appropriate range for your piece of equipment (e.g. if the conjugate is at 20 OD and is diluted 1:20 the Abs_{max} for a 1 cm light path is expected to be around 1 AU).

TECHNICAL SUPPORT

For technical enquiries get in touch with our technical support team at www.expedeon.com/contact

For further information see our website: www.expedeon.com

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