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# Ab154873
# Gold Conjugation Kit (40 nm, 20 OD)

## A product of Expedeon, an Abcam company

Applicable to Expedeon product codes 230-0005, 230-0010, 2300015.

For the Conjugation of Antibodies or Proteins to Gold (40 nm, 20 OD).

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This product is for research use only and is not intended for diagnostic use.

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## 1. Overview

The Abcam Gold Conjugation Kit (40 nm, 20 OD) (ab201808) allows antibodies or proteins to be covalently attached to ultra-stable Gold nanoparticles at very high OD quickly and easily. The Abcam Gold nanoparticles have a protective surface coat that can withstand the most extreme conditions (e.g. 2.5M NaOH at 70°C for > 1 hour).

Gold nanoparticles in this kit are freeze dried. The conjugation reaction is initiated simply by reconstituting the freeze-dried Gold nanoparticles with the antibody, which becomes attached (through lysine residues) to the surface of the Gold nanoparticles.

The hands-on time for the Abcam Gold conjugation procedure is around 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.

The resulting covalent conjugates are more stable than those prepared by passive adsorption methods. Moreover, unlike passive methods, the coating process is independent of the isoelectric point of the antibody, avoiding the need for extensive trials at different pH values. All antibodies can be labelled at a single pH.

## 2. Materials Supplied and Storage

Store the kit at -20ºC upon receipt. The quencher, reaction buffer and antibody diluent can be stored at either +4°C or -20°C.

| Item | Quantity | | | Storage temperature |
|---|---|---|---|---|
| | **3 x 1 µg** | **10 x 1 µg** | **1 x 10 µg** | |
| Gold 40 nm | 3 vials | 10 vials | 1 vial | -20°C |
| Gold 40 nm Reaction Buffer | 1 vial | 1 vial | 1 vial | -20°C or +4°C |
| Gold 40 nm Antibody Diluent | 1 vial | 1 vial | 1 vial | -20°C or +4°C |
| Gold 40 nm Quencher | 1 vial | 1 vial | 1 vial | -20°C or +4°C |

Reagents are ready to use as supplied.

# 3. Technical Considerations

## 3.1 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)* | Yes |
| Sugars | Yes |
| Glycerol | < 50% |
| PBS* | No |
| Thiomersal | No |
| Thimerosal | No |
| Merthiolate | No |
| Sodium Azide | No |
| BSA | No |
| Gelatin | No |
| Tris | No |
| Glycine | No |
| Carboxylic acids (e.g. EDTA, Citrate) | No |
| Nucleophilic components *(Primary amines e.g. amino acids or ethanolamine and thiols e.g. mercaptoethanol or DTT)* | No |

***** *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.*

***Δ Note:*** *If the antibody is not in a suitable buffer for conjugation, please consult our* [*Antibody Purification Kits*] page.

**3.2 Amount and Volume of antibody to conjugate:**

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.

| Kit size | Recommended initial amount of antibody |
|---|---|
| 3 x 1 µg | 1 µg |
| 10 x 1 µg | 1 µg |
| 1 x 10 µg | 10 µg |

## 4. Assay Procedure

**4.1** Allow all of the reagents to warm to room temperature.

**4.2** Dilute your stock antibody with the antibody diluent provided in the kit to 0.1 mg/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.*

**4.3** For each reaction add the reaction buffer and your now diluted antibody according to the table below and mix gently:

| Item | 3 x 1 µg | 10 x 1 µg | 1 x 10 µg |
|---|---|---|---|
| Diluted Antibody | 12 µL | 12 µL | 120 µL |
| Gold 40 nm Reaction Buffer | 42 µL | 42 µL | 420 µL |

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

**4.4** Transfer 45 µL of the mixture to a vial of Gold 40 nm If using the 3 or 10 x 1 µg kits or 450 µL of the mixture if using the large volume kit (1 x 10 µg). Reconstitute the Gold 40 nm by gently pipetting up and down. Leave the reaction for 15 minutes at room temperature.

**4.5** After 15 minutes, add the appropriate volume of Gold 40 nm Quencher according the table below to stop the reaction. Mix well, but gently.

| Item | 3 x 1 µg | 10 x 1 µg | 1 x 10 µg |
|---|---|---|---|
| Gold 40 nm Quencher | 5 µL | 5 µL | 50 µL |

**4.6** Leave the reaction for 5 minutes. You now have 20 OD conjugate (50 µL for 3 and 10 x 1 µg kits, and 500 µL for 1 x 10 µg kit). Dilute further as required for your application.

*◻ **Note**: For a conjugate 100% free from unbound antibody we recommend washing the particles by adding 10 times the volume of the 1:10 diluted quencher to the conjugate (i.e. 1 mL to 100 µL of conjugate) and then centrifuge it in a microfuge at: 9,000 x g for 10 minutes. Carefully remove the supernatant, gently tap the pellet and add 1:10 diluted Gold 40 nm Quencher for long term storage in the fridge (up to 1 year), or 1:10 diluted Gold 40 nm Quencher with addition of 0.5 - 2% BSA for lateral flow assays (LFA) or your preferred*

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

### 4.7 Conjugated Antibody Storage:

Once the antibody has been labelled, we recommend storing the conjugate at +4°C. The Gold 40 nm Quencher added in the last step of the conjugation 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 overall stability of the conjugate will be determined by the stability of the antibody itself, as it will be first to degrade. Provided that your antibody is stable, the conjugate will also be stable.

### 4.8 Measuring conjugate concentration:

The maximum absorbance (Absmax) for 40 NM Gold 530 nm.

To determine the effective concentration of the conjugate obtained we advise to measure the Absmax 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 Absmax for a 1 cm light path is expected to be around 1 AU).

## 5. Frequently Asked Questions

### 5.1 What is different about Gold nanoparticles?

Gold nanoparticle products are 'conjugation friendly' nanoparticles with a proprietary surface coat that greatly enhances Gold stability and permits easy covalent attachment of a variety of molecules, including antibodies, analytes and other biomolecules.

### 5.2 Does the antibody bind to the metal surface?

No. The protective surface coat completely shields the metal surface and prevents direct metal-antibody interactions. For

this reason, you cannot use Gold nanoparticles for passive conjugation of antibodies.

**5.3 Can antibodies from different species be used?**

Yes, the Gold system has been tested with antibodies from a variety of species including mouse, rabbit, goat and sheep.

**5.4 What type of linkage to gold is formed?**

The antibody becomes covalently and irreversibly attached via lysine residues to the Nanoparticle's surface.

**5.5 Can antibody fragments be conjugated?**

Yes. One of the advantages of the protective coat is that it is less likely than a bare metal surface to cause denaturation and loss of affinity of the antibody fragments.

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