ab188215
GOLD Conjugation Kit
(20nm, 20OD)

Instructions for Use

For the Covalent Conjugation of Antibodies or Proteins to Gold

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

Abcam’s GOLD Conjugation Kit (20nm, 20OD) (ab188215) allows antibodies or proteins to be covalently attached to ultra-stable* GOLD nanoparticles at very high OD quickly and easily. The hands-on time for the Gold conjugation procedure is about 2 minutes and the conjugate is ready to use within 15 minutes.

*Gold has a protective surface coat that can withstand the most extreme conditions (e.g. 2.5M NaOH at 70°C for >1 hour).

The Gold nanoparticles in this kit are supplied as a freeze dried mixture. The conjugation reaction is initiated simply by adding a solution of the antibody, which becomes attached (via lysine residues) to the gold surface.

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, and extensive trials at different pH values are not required; all antibodies react at a single fixed pH.

The 3 and 10 test Conjugation Kits are designed to label 12 µl per vial.

The 1 test Conjugation Kit is designed to label 120 µl per vial.
2. Kit Contents

<table>
<thead>
<tr>
<th>Components</th>
<th>Amount</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 Tests</td>
<td>10 Tests</td>
</tr>
<tr>
<td>Amount of Antibody/Test</td>
<td>12 µl</td>
<td>12 µl</td>
</tr>
<tr>
<td>Gold 20nm</td>
<td>3 vials</td>
<td>10 vials</td>
</tr>
<tr>
<td>Gold 20nm Reaction Buffer</td>
<td>1 vial</td>
<td>1 vial</td>
</tr>
<tr>
<td>Gold 20nm Antibody Diluent</td>
<td>1 vial</td>
<td>1 vial</td>
</tr>
<tr>
<td>Gold 20nm Quencher Reagent</td>
<td>1 vial</td>
<td>1 vial</td>
</tr>
</tbody>
</table>

3. Storage and Handling

For storage temperatures please see the Table above

For handling refer to Safety Datasheet
4. Additional Materials

Microfuge Tubes (0.5 or 1.5 ml)

Microfuge

5. General Guidelines

A. Prior to Labeling

- Antibody must be purified
- Avoid protein additives (e.g. BSA)
- Avoid amino acids (e.g. glycine)
- Avoid other primary amines (e.g. Tris)
- Avoid thiols (e.g. mercaptoethanol, DTT)
- Avoid carboxylic acids (e.g. EDTA, citrate)

You should pay particular attention to the detail on the data sheet supplied with your antibody. If you are in any doubt about the suitability of the buffer/additives in your preparation of antibody please dialyse against a suitable buffer (see FAQs) or contact the Abcam technical team for further advice.
B. Amount 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.

<table>
<thead>
<tr>
<th>Amount of antibody for 3 or 10 test kit</th>
<th>5 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount of antibody for 1 test kit</td>
<td>50 µg</td>
</tr>
</tbody>
</table>
6. Labeling Protocol

*Note:* The following protocol is for 1 vial from the 3 or 10 test Conjugation Kit. Adjust volumes as required for the larger 1 test Conjugation Kit.

1. Allow all of the reagents to warm to room temperature.
2. Dilute your stock antibody with the Gold 20nm Antibody Diluent provided in the kit to 0.5 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. In order to vary the amount of antibody added, you must change the concentration of the stock antibody and use a fixed volume.*
3. For each reaction: In a clean 0.5 ml or 1.5 ml microfuge tube add 42 µl of Gold 20nm Reaction Buffer and then 12 µl of your diluted antibody from Step.2. Mix thoroughly.
   *(Note: 420 µl Gold 20nm Reaction Buffer and 120 µl antibody for large volume kit).*
4. Transfer 45 µl of the mixture to a vial of Gold 20nm. Reconstitute the freeze dried mixture by gently pipetting up and down. Allow to stand at room temperature for 15 minutes.
   *(Note: 450 µl of the mixture to a vial of Gold 20nm for large volume kit).*
5. Add 5 µl of Gold 20nm Quencher Reagent and mix gently. Leave the reaction for 5 minutes. You now have 50 µl of 20 OD conjugate. Dilute further as required for your application. (Note: 50 µl Gold 20nm Quencher Reagent for large volume kit – final volume 500 µl of 20 OD conjugate).

Notes:

a) While you should avoid thiols (e.g. DTT or mercaptoethanol) the other interfering substances noted in section 5 have no negative effect once the conjugate has been formed.

b) For a conjugate 100% free from unbound antibody we recommend to wash the particles adding 20 times the volume of the 1:10 diluted Gold 20nm Quencher Reagent to the conjugate (i.e. 1 ml to 50 µl of conjugate) and then centrifuge it in a microfuge at 9,000 x g for 20 min. Carefully remove the supernatant, gently tap the pellet and add 1:10 diluted Gold 20nm Quencher Reagent for long term storage in the fridge (up to 1 year) or 1:10 diluted Gold 20nm Quencher Reagent 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).

Gold 20nm Labeled antibody should be stored at +4°C.
7. Frequently Asked Questions

What can I do if my antibody preparation contains interfering substances?

Unless the antibody contains contaminating proteins, the simplest procedure is to dialyse the antibody against a suitable buffer. Alternatively, desalting columns may be used. Relatively weak buffers (e.g. 20mM) are strongly preferred so that the pH conditions of the covalent reaction are not significantly altered upon addition of the antibody. MES, MOPS, HEPES are preferred dialysis buffers. If you have contaminating proteins in your antibody (e.g. BSA), you will need to purify the antibody before use.

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 Conjugation Kits for passive conjugation of antibodies.

Can antibodies from different species be used?

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

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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