ab154876

Gold Conjugation Kit

(80nm, 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.

Version 2 Last Updated 4 April 2019

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1. Introduction

Abcam's Gold Conjugation Kit 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 20 minutes.

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

Components	Amount			Storage
	3 Tests	10 Tests	1 Test	
Amount of Antibody/Test	**12 µl**	**12 µl**	**120 µl**	
Gold Reaction Buffer	1 vial	1 vial	1 vial	-20°C
Gold Antibody Diluent	1 vial	1 vial	1 vial	-20°C
Gold	3 vials	10 vials	1 vial	-20°C
Gold Quencher	1 vial	1 vial	1 vial	-20°C

3. Storage and Handling

For storage temperatures please see the Table

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 amino acids (e.g. glycine)
- Avoid other primary amines (e.g. Tris)
- Avoid thiols (e.g. mercaptoethanol, DTT)
- Avoid carboxylic acids (e.g. EDTA)

Stock antibodies at concentrations of >1mg/ml are recommended; as this allows potentially interfering substances in the antibody preparation to be diluted out (see Step 6.2). You should pay particular attention to the composition of the stock antibody if you require less than a 1 in 5 dilution in step 6.2 of the protocol. If you are in any doubt about the suitability of buffers/additives in your preparation of antibody please contact our technical team for advice.

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

B. Amount of Antibody

The optimum amount of antibody (which will influence the number of antibody molecules per particle) may be application-dependent and you may need to conjugate different amounts of antibody to optimize your assay. The initial amount of antibody recommended corresponds to 10 µg antibody per ml of 10 OD gold, which is about half of that normally used for passive (non-covalent) conjugations. However lower or higher concentrations can be explored as there is no risk of aggregation because of the protective surface coating.

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 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 at 0.05 and 0.2 mg/ml in the first instance. 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 reaction buffer and then 12 µl of your diluted antibody from Step.2. Mix thoroughly.
   (Note: 420 µl Gold reaction buffer and 120 µl antibody for large volume kit).
4. Transfer 45 µl of the mixture to a vial of GOLD. Reconstitute the freeze dried mixture by gently pipetting up and down. Allow to stand at room temperature for 15 minutes.
   (Note: 450 µl Gold for large volume kit).
5. Add 5 µl of Gold Quencher 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 Quencher for large volume kit – final volume 500 µl of 20 OD conjugate).

Note: *For a conjugate 100% free from unbound antibody we recommend washing the particles and 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 9,000 g 10 minutes. 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.*

Note: *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.*

Gold Labeled antibody should be stored at +4°C

7. Frequently Asked Questions

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

The simplest procedure is to dialyse the antibody against a suitable buffer. Relatively weak buffers (e.g. 20mM) are preferred so that the pH conditions of the covalent reaction are not significantly altered upon addition of the antibody. See also the comments in Section 5 of the protocol. If dialysis is not practical you can use other popular methods of buffer exchange if required e.g. desalting columns.

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.

What type of linkage to gold is formed?

The antibody becomes covalently and irreversibly attached via lysine residues to the GOLD surface.

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.

What if I need bulk material?

The kit that you have purchased is a convenience product for rapid production of small quantities of conjugate for screening purposes. Abcam offers the kit in a range of sizes. Please enquire.

Is the kit suitable for conjugating analytes and other small molecules?

Yes, but please contact technical services. Depending on the functional groups on your analyte you may need a different type of surface or different chemistry for optimal results.

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

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