BioTeZ

Gold Conjugation Kit

For Gold-labelling of proteins/antibodies

Instructions

Reagents for coating a Gold-Label to target proteins/antibodies

Product Code: BTGCK-001

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Note: Read the instructions carefully before using the reagents!

1. Intended Use

This Gold Conjugation Kit's intended use is for the labelling of proteins, such as antibodies, with gold nano particle (size 40 nm) while the protein is retaining a high degree of specific activity and can be used for evaluation of its interaction with other biomolecules.

2. Description

Proteins bind adsorptive to gold nano particles. For preparing stable gold conjugates and for retaining of proteins binding characteristics, the coating pH value should be slightly above the isoelectric point of the protein. The titration of the pH will help to find the best coating pH value.

This kit allows to screen coating between pH 5.2 and pH 10.5. If you know roughly the isoelectric point (for proteins: http://isoelectric.org/) you can leave out not relevant tests and save materials.
The stability of the gold conjugates is tested in the presence of salt (Stability Test Solution). The Block Solution stops the conjugation reaction.

After screening and identifying the optimal pH value for gold coating you can proceed to coat 50 µg of your desired antibody/protein or you could conduct further tests to identify the optimal gold-protein ration, which fits best for your application.

Options for a double labelling strategy with your antibody/protein by using TA-PAS Labelling Kit: The BioTeZ TA-PAS Labelling Kit allows many possibilities to modify and optimize your immunoassay. The synthetic small TA label binds fast and highly efficient to the PAS capture antibody. If using the BioTeZ TA-PAS Labelling Kit, do the coupling of the TA label first (see our TA-PAS Labelling Kit # BTTAP-001) and then continue with gold coating procedure as written in this instruction.

The optimal pH value of unmodified IgG1-antibodies is usually around pH 8.8 ± 0.3 and around pH 7.6 ± 0.4 for TA-PAS labelled IgG1-antibodies.

3. Storage and Components

The **Gold labelling kit** is shipped at 2-8°C and stored as the following: the Gold Sol at room temperature (20-26°C) and the rest of solutions at 2-8°C (see the table below).

Table: Overview of kit contents and storage conditions

Components	Quantity (Volume)	pH	Storage temp.
Buffer #1	100 µl	10.50	2-8 °C
Buffer #2	100 µl	9.35	2-8 °C
Buffer #3	100 µl	9.03	2-8 °C
Buffer #4	100 µl	8.80	2-8 °C
Buffer #5	100 µl	8.63	2-8 °C
Buffer #6	100 µl	8.30	2-8 °C
Buffer #7	100 µl	7.91	2-8 °C
Buffer #8	100 µl	7.59	2-8 °C
Buffer #9	100 µl	7.30	2-8 °C
Buffer #10	100 µl	6.65	2-8 °C
Buffer #11	100 µl	5.92	2-8 °C
Buffer #12	100 µl	5.55	2-8 °C
Buffer #13	100 µl	5.21	2-8 °C
Stability Test Solution	100 µl	-	2-8 °C
Block Solution	100 µl	-	2-8 °C
Gold Sol (size of the particles: 40 nm)	2300 µl	-	**20-25°C**
reaction test tubes	30 tubes of 1.5 ml		
reaction test tubes	1 tube of 2 ml		

Required equipment and materials not included in the kit:

- Vortex Mixer
- Pipettes
- 80 µg protein/antibody solution (1 mg/ml) in **15 mM K-PO_4, pH 7.4, 50 mM NaCl (= 0.3 × PBS)**

4. Considerations prior to Coating/Labelling

- Use precaution to avoid contamination when handling the Gold Sol; please work aseptic
- Do not freeze unconjugated Gold Sol
- Antibody solution for coating may not contain sulphur containing reagents e.g. Proclin 950 (concentration should be less than 0.1%), thimerosal
- Avoid thiols e.g. DTT, cysteine
- No BSA or Gelatin
- Some enzymes can lose their activity because of labelling

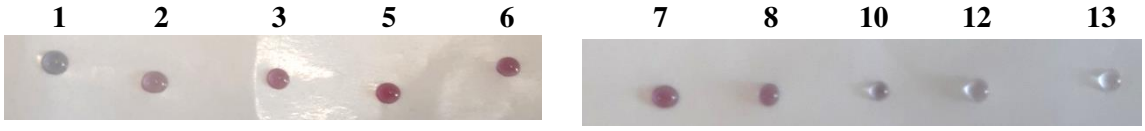
5. Sample Preparation

5.1 Concentration and optimal pH conditions

The antibodies/proteins used with this kit must be at a concentration of 1 mg/ml and should be in a 0.3 × PBS buffer solution.
If they are not in a 0.3 × PBS buffer solution, change the buffer of the protein against 0.3 × PBS by using ultrafiltration (30-50 kDa for antibodies) or dialysis (high ion strength will lead the unconjugated Gold Sol to aggregate). Be aware that after dialyse, the concentration of your antibody/protein could change (normally it is decreased).

1. Place 1-13 tubes in a rack and label them with the numbers 1-13 according to the provided buffers 1-13.
2. Add 2 µl of a 1 mg/ml antibody/protein solution to each tube.
3. Add 2 µl of the provided buffers, from # 1 till # 13 and mix thoroughly (about 2-3 seconds).
4. Re-suspend the Gold Sol and add 60 µl of Gold Sol in each of the 1-13 tubes. Mix under vortex (about 5-10 seconds).
5. Incubate the reaction mixes for 1 h at room temperature (20-25 °C).
6. After 1 h incubation, but before stopping the reaction, make a **Stability-Test:**
   a. Take new reaction tubes and combine 2 µl of each reaction mix tube (1-13) with 2 µl Stability Test Solution.
   b. Sols with incomplete binding will form aggregates (turn grey), while completely coated sols will remain stable (red). The aggregated Gold Sols are not suitable for use in immunoassays.

c. For further testing and large scale coating, choose the mix tube with only a slight change in colour. See the example below.



d. In this example, the chosen sample is number 5, it means buffer #5 (pH 8.63) will be further used in order to label a big amount (up to 50 µg) of the protein/antibody.

e. After identification of the optimal coating pH value, all not needed test tubes could be discarded.

7. Proceed with the selected tubes and conduct the stop reaction by adding 2 µl **Block Solution** and incubate over night at room temperature (20-25 °C).

NOTE: This protocol may be modified or scaled as needed. When developing a new assay, it is recommended to determine the optimal antibody-gold ratio. Therefore, it is recommended to decrease and increase the amount of antibody added to the Gold Sol. Often, a 20% increase or decrease in antibody/protein added is sufficient to find out if better conjugate characteristics appear. A few cases require a 40% or more increase or decrease to find the optimal Gold protein ratio.
A sensitive lateral-flow assay requires that all of the antibody that is added to the Gold Sol is irreversibly bound to the gold particles. Any free antibody serves to short-circuit the assay. This behaviour ultimately sets the sensitivity limits of an assay.

5.2 Up-scaling of the Gold labelling

1. Take 50 µl (50 µg) antibody/protein
2. and mix thoroughly with 50 µl of the chosen buffer (about 2-3 seconds)
3. Re-suspend the Gold sol and add 1.5 ml Gold sol on antibody/buffer mix
4. Mix using vortex (about 5-10 seconds)
5. Incubate the reaction mix for 1 h at room temperature (20-25 °C).
6. Check once more with the Stability Test Solution, if the labelling has worked: 2 µl mix and 2 µl Stability Test Solution
7. Add 50 µl Block Solution and mix again and incubate over night at 20-25 °C

NOTE: If you need to label less than 50 µg protein, please adjust to the suitable amount of Buffer, Gold Sol and Block Solution.

Related products

Product	Cat. No.	Package Size
BioTeZ Peroxidase Labelling Kit Preparation of HRP labels using highly activated Poly-HRP	BTHRPK-03 BTHRPK-05	For 3 labels For 5 labels
Biotin Labelling Kit Reagents for the biotinylation of 1 – 10 mg antibody / 1-5 mg other biomolecules	BTBIOK-05 BTBIOK-10	For 5 labels For 10 labels
TA-PAS Labelling Kit (for immobilization and enrichment of biomolecules) Alternative to or in combination with Biotin/Streptavidin	BTTAP-001	For 1 label
PAS-Gold PAS capture antibody gold labelled (40nm)	20 500 005 20 500 020	0.5 ml 2.0 ml
PAS-HRP PAS capture antibody HRP labelled	20 600 001	100 µg
PAS-Poly-HRP PAS capture antibody Poly-HRP labelled (40nm)	20 700 001	100 µg
Polystreptavidin E (Polystrept E) Very high Biotin binding capacity; optimized for using biotin-streptavidin in combination with TA-PAS system in lateral flow tests	10 121 010 10 121 010 10 121 010	1 mg 5 mg 10 mg
Recombinant Streptavidin, FITC-labelled	20 400 001	100 µg
Recombinant Streptavidin, Poly-HRP-labelled	20 201 001	100 µg
Recombinant Streptavidin	10 110 010 10 110 050 10 110 100	1 mg lyophilized 5 mg lyophilized 10 mg lyophilized
Polystreptavidin R (Polystrept R) Very high Biotin binding capacity	10 120 010 10 120 050 10 120 100	1 mg 5 mg 10 mg
Streptavidin-coated 8-well strip plate, C well, clear Polystyrene	F8PS-CL-SC	5 plates
Polystreptavidin-coated 8-well strip plate C well, clear Polystyrene	F8PS-CL-MC	5 plates
Labelled Streptavidin Poly HRP, HRP, Gold, FITC, EU, Carbon Black		
Streptavidin coating kits	**Cat. No.**	**Package Size**
BioTeZ Streptavidin Coating Kit For Streptavidin Coating on beads, chips, membranes etc.	BTCK-SC0125 BTCK-SC0500	1 Kit/ 125 ml solution 1 Kit/ 500 ml solution
BioTeZ Polystreptavidin R Coating Kit For Polystreptavidin R Coating on beads, chips, membranes etc.	BTCK-MC0125 BTCK-MC0500	1 Kit/ 125 ml solution 1 Kit/ 500 ml solution
BioTeZ Polystreptavidin R Coating Kit Glass For Polystreptavidin R Coating on glass	BTCKG-MC0125 BTCKG-MC0500	1 Kit/ 125 ml solution 1 Kit/ 500 ml solution

Important points and precautions

1. The manufacturer assumes no liability and indicates that the user is solely responsible for the consequences of any alterations made, for non-observance of instructions or for performing the coating procedure without paying due attention.
2. The equipment used must be maintained in accordance with the manufacturers' instructions and any applicable guidelines. Before equipment is used it should be checked for fault-free operation.
3. Excess reagents that have exceeded their expiry date/lifetime should be disposed of correctly. You should observe the regulations that apply to you.
4. The reagents may only be carried out by trained specialists. The production steps are validated for use at the indicated temperature. Deviations in the climatic conditions can negatively influence the results.
5. Ensure that the materials, equipment and reagents are clean, paying particular attention e.g. to vessels and pipette tips.
6. Observe general health and safety regulations.
7. Follow the instructions for this test very closely.
8. When disposing of the reagents, observe any potential harm they may cause to the environment. Observe the regulations that apply to you.
9. Observe safety regulations, e.g. do not eat, drink or smoke in the workplace; keep materials and reagents away from foods and feeding stuffs; wear protective clothing (lab coat, safety glasses and gloves).
10. Never carry out pipetting operations using the mouth; always use suitable equipment or devices.

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