Naked Gold Conjugation Kit

Applies to REF NGIB18-1, NGIB18-2, NGIB18-3

This is a protocol for conjugation of antibodies with the Naked Gold Conjugation Kit. It contains specific guidelines regarding the optimization and application of the gold conjugates with the gRAD system (http://www.bioporto.com/gRAD-procedures.aspx).

Introduction

Naked Gold Conjugation Kit is an easy-to-use kit for preparing highly reactive gold conjugates. The Naked Gold Conjugation Kit enables users to quickly determine (in about one hour) the optimal colloidal gold binding conditions for coating the Fc portion of purified monoclonal and polyclonal antibodies. The resulting gold particles are highly active and greatly increase the sensitivity of lateral-flow tests. The Naked Gold Conjugation Kit helps to determine the ideal pH for coating the gold particles with antibodies.

Kit Content

The Naked Gold Conjugation Kit (REF NGIB18-1, NGIB18-2 and NGIB-3) contains

REF	Contents	Quantity
NGIB18-1	Naked Gold Conjugation Kit (20 nm and 40nm):	1 box
	1(20) Naked Gold Sol 20 nm*	9 mL
	1(40) Naked Gold Sol 40 nm	9 mL
	2 Buffer solution A – Cap with black dot	1 mL
	3 Buffer solution B – Cap with green dot	1 mL
	4 Buffer solution C – Cap with blue dot	1 mL
	5 Buffer solution D – Cap with red dot	1 mL
	6 Stabilizing buffer – Green cap	2 x 2 mL
NGIB18-2	Naked Gold Conjugation Kit (20 nm):	1 box
	1(20) Naked Gold Sol 20 nm	2 x 9 mL
	2 Buffer solution A – Cap with black dot	1 mL
	3 Buffer solution B – Cap with green dot	1 mL
	4 Buffer solution C – Cap with blue dot	1 mL
	5 Buffer solution D – Cap with red dot	1 mL
	6 Stabilizing buffer – Green cap	2 x 2 mL
NGIB18-3	Naked Gold Conjugation Kit (40 nm):	1 box
	1(40) Naked Gold Sol 40 nm	2 x 9 mL
	2 Buffer solution A – Cap with black dot	1 mL
	3 Buffer solution B – Cap with green dot	1 mL
	4 Buffer solution C – Cap with blue dot	1 mL
	5 Buffer solution D – Cap with red dot	1 mL
	6 Stabilizing buffer – Green cap	2 x 2 mL

*A sol denotes a colloid solution of solid particles i.e. gold particles

Materials required but not provided

- 2 mL screw cap tubes, (Axygen SCT-200-SS-C or similar can be used)
- Pipettes and tips

Storage of the Kit and its components

The kit and the components should be stored at 2 – 8°C. Do not freeze the Gold sol as it will aggregate. Bring reagents to room temperature before using.

Principle of conjugation of antibodies with Naked Gold

Gold colloids that bind ligands through a sulphur bond have been proven to be highly successful for application with lateral-flow assays. If the antibodies to be conjugated do not display a suitable available number of thiol groups (-SH) to promote bonding to the gold particles, they will bind exchangeably with the gold particles through ion-exchange interactions. Such antibodies do not form stable gold sols that are suitable for lateral flow assays.

The Naked Gold Sol is negatively charged and nanogold particles remain in solution because they repel each other. If the coating pH is too low, the antibodies will have a net positive charge and cause the Naked Gold Sol to aggregate. If the pH is too high, the protein will have a net negative charge and will be repelled from the gold particles. Therefore, for optimal binding of the antibody to the gold, while retaining a high degree of specific activity, the coating antibody should be mostly neutral but not positively charged. To achieve this the coating pH should be slightly above the isoelectric point of the antibody. This is the challenge of preparing stable gold conjugates. In a few cases, the titration of the pH may need to be fine-tuned.

This kit allows to screen coating pH-values between 5.4 and 10.1 by mixing varying amounts of buffers A, B, C and D contained in the kit. The resulting mixtures are then combined with the antibody to adjust its net charge before addition to the Naked Gold Sol. It is important to add the antibody to the sol with mixing to ensure an even coat distribution. The stability of the gold conjugates is tested in the presence of salt, see point 3.2 below. The Stabilizing buffer stops the conjugation reaction.

Precautions

1. For Research Use only. Not for use in diagnostic procedures.
2. This kit should only be used by qualified laboratory staff.
3. Do not freeze unconjugated Naked Gold Sol.
4. High ion strength will lead the unconjugated Gold Sol to aggregate.
5. Antibody solutions for coating may not contain sulphur containing reagents e.g. Proclin 950.
6. Do not contaminate the Naked Gold Sol when handling it.
7. Avoid release into the environment. Dispose containers and unused contents in a safe way and in accordance with national and local regulations.

Protocol

1. Sample Preparation

Prepare a solution of the chosen antibodies with a concentration of 1 mg/mL or greater. The solution must optimally be prepared in ultra high purity water. In some applications, a low salt buffer may be possible – not exceeding 25 mmol/L salt).

The gold particles should be saturated with antibody for optimal stability. The saturation point for 20 nm gold is about 60-70 µg antibody/mL of Naked Gold Sol. For the 40 nm gold, this point is typically near 30 µg antibody/mL of Naked Gold Sol. The presence of salt in the solution will interact and destabilize the negatively-charged gold particles, affecting the apparent optimal coating pH.

2. Gold conjugation with antibodies

Note: **Use precautions to avoid contamination when handling the Naked Gold Sol**

2.1. Label two sets of tubes with the pH value from the provided pH chart (or number them 1 through 10).
2.2. Shake or swirl the gold from the kit to resuspend any settled material. Place 0.5 mL Naked Gold sol into each tube of one of the sets of 10 tubes.
2.3. Add the indicated volumes of the buffers to the second set of tubes, as outlined in the pH chart.

pH chart

Tube #	pH	Buffer A	Buffer B
1	5.4	9 µL	1 µL
2	6.6	8 µL	2 µL
3	7.3	6 µL	4 µL
4	7.8	4 µL	6 µL
5	8.2	2 µL	8 µL

Tube #	pH	Buffer C	Buffer D
6	8.4	10 µL	0 µL
7	8.8	8 µL	2 µL
8	9.2	6 µL	4 µL
9	9.6	4 µL	6 µL
10	10.1	2 µL	8 µL

2.4. Transfer the appropriate volume of antibody solution (See Sample Preparation Section), to each buffer solution. Mix and then reaspirate into the pipette tip.
2.5. Add the antibody/buffer solution to the appropriately labelled tube containing the Naked Gold Sol under vortex mixing.
2.6. Mix for 5-10 seconds.
2.7. Allow the reaction to continue for a total of 30 minutes.
2.8. Make a selection of successful conjugations following the guidelines in section 3. For those selected, stop the reaction by the addition of 100 µl of Stabilizing buffer and mix for a further 30 minutes.

3. Choosing and testing the gold conjugates to use with gRAD strips (Cat. No. gRAD(1)-120)

3.1 A simple visual inspection of the colour of the conjugation mixtures in the 10 tubes described above will help to select the successful conjugations. If the pH is too low pH the gold sol will aggregate and the conjugation mixture will change colour to a darker shade. The aggregated gold sols are not suitable for use in immunoassays and should be discarded. Choose the mixtures with only a slight change in colour for further testing.
3.2 In order to test the effectiveness of the conjugation reaction, a salt test can be performed. For each tube selected in point 2.8, transfer 10 µl of coated gold sol to

10 µL of 1 mol/L NaCl solution. Sols with incomplete coating will fall out of solution (turn black), while completely coated sols will remain stable (red). This happens within 60 seconds.
3.3 The chosen conjugate(s) according to point 3.1 and 3.2 is/are now ready for use with the gRAD strips at nominal usage of 2-5 µL per assay. This volume typically gives the best compromise between assay sensitivity, robustness and background.

In some conjugates that result in non-specific reactivity (seen as background when performing the gRAD), it is often best to allow the blocker (Stabilizing buffer) to react for an additional 16 hours at room temperature.

NOTE: This protocol may be modified or scaled as needed. When developing a new assay, it is important to determine the optimal amount of antibody to add to the Naked Gold Sol. Once the tubes have been assayed, it is useful to further optimize binding by both decreasing or increasing the amount of antibody added to each tube. Often, a 20% increase or decrease in antibody or protein added is sufficient to yield an optimal coating procedure. A few cases require a 40% or more increase or decrease in coating antibody.

A sensitive lateral-flow assay requires that all of the antibody that is added to the Naked 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.

Stability of conjugated gold
Gold particles coated with antibodies and stabilized are stable in solutions of high ionic strength and can be frozen. It is recommended to keep the concentration of the Stabilizing buffer higher than 10% in any resulting test reagent.

The stability of the gold conjugates must be ascertained by the user. In general, optimally coated gold is stable for 2 weeks at 2-8°C. It is recommended to store the gold conjugates frozen in aliquots.

Liability
This Kit is only intended for use by qualified personnel carrying out research activities. If the recipient of this test passes it on in any way to a third party, this instruction must be enclosed, and said recipient shall at recipient's own risk secure in favour of BioPorto Diagnostics A/S all limitations of liability herein.

Symbols

REF	Catalogue number		Do not reuse
LOT	Batch code		Manufacturer
	Consult instructions for use	8°C 2°C	Temperature limitation
	Use by		

Related products:

REF	Product Name	Quantity
gRAD(1)-120	gRAD OneDetection – 120 strips	6 x 20 strips
gRAD(1)-Kit	gRAD OneDetection – Kit	1 Kit
SDB50	Sample Dilution Buffer	50 mL

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