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ab269902
Gold Conjugation Kit (20 nm, Maleimide)

A product of Expedeon, an Abcam company

Applicable to Expedeon product codes: 271-0005, 271-0015

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Gold Conjugation Kit (20 nm, Maleimide) datasheet:
www.abcam.com/ab269902
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For the Covalent Conjugation of biomolecules to Gold Nanoparticles.

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

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1. Overview

Gold Conjugation Kit (20 nm, Maleimide) (ab269903) provides an easy-to-use procedure that allows researchers to covalently label proteins, peptides, oligonucleotides and other biomolecules containing thiol groups (-SH) with ultra-stable Gold nanoparticles. Maleimide groups are attached to the proprietary surface coat, which does not detach from gold surfaces even under the most extreme conditions. The hands-on time for the conjugation procedure is 5 minutes and the conjugate is ready to use within 1 hour.

The antibody to be labeled should be purified, in an appropriate buffer for conjugation and at a suitable concentration, as described in the Technical Consideration section. If not, consider using our antibody purification and concentration kits.

The conjugation reaction is initiated by adding a solution of the thiol-activated molecule (protein, peptide, antibody, antibody fragment or oligonucleotide) to the Gold-Maleimide nanoparticles.

2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Avoid repeated freeze-thaws of reagents.

Item	3 x 5 µg	1 x 50 µg	Storage temperature
Gold mix	3 vials	1 vial	-20°C
Reaction Buffer	1 vial	1 vial	-20°C
Quencher reagent	1 vial	1 vial	-20°C
Diluent Reagent	1 vial/bottle	1 vial/bottle	-20°C

Δ Note: *Once the kit is opened, unused vials should be stored with silica gel (the vials are moisture sensitive).*

3. Technical considerations

3.1 Pre-Conjugation Considerations:

Disulfide bonds in protein structures (e.g. between cysteines) must be reduced to thiol groups for them to react with maleimide reagents. Alternatively, thiol groups must be introduced into molecules that have no indigenous thiol groups.

Free thiols (e.g. β-mercaptoethanol, DTT) must be excluded from samples and maleimide reaction buffers by desalting, because they will compete for coupling sites on the gold nanoparticles; a desalting step post reduction is mandatory if thiols are used to reduce the disulphide bonds.

Single stranded oligonucleotides must be between 10 and 120 bases in length and contain a terminal thiol group, which must

be added during synthesis. All commercial oligo suppliers offer this modification. The efficiency of conjugation is slightly higher with 5' aminated oligos.

Oligonucleotides should be HPLC purified; this is not necessary for proteins or antibodies.

The reducing agent TCEP is not compatible with the kit.

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

3.2 Amount of biomolecule:

The optimum amount of biomolecule (which will influence the number of molecules per particle) may be application-dependent and must be determined by experimentation; you may need to conjugate different amounts of biomolecule to optimize your assay.

For example, around 5 µg of thiol-activated IgG antibody are likely to give optimal results for the 3 x 5 µg kit. The suggested antibody amount for the 1 x 50 µg kit is exactly 10 times the one optimized for the 3 x 5 µg kit (e.g. 50 µg for 20 nm).

The recommended starting concentration for oligonucleotides is >100 µM, as this allows potentially interfering substances in the oligonucleotide preparation to be diluted out.

The recommended volume in which the biomolecule is added is 45 µl (3 x 5 µg kit) and 450 µl (1 x 50 µg kit).

4. Assay Procedure

– Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.

4.1 Dilute the biomolecule in the Diluent Reagent to produce a final volume of 40 µL (3 x 5 µg kit) or 400 µL (1 x 50 µg kit).

4.2 Add 5 µL (3 x 5 µg kit) or 50 µL (1 x 50 µg kit) of Reaction Buffer. Mix gently.

4.3 Remove the cap from the vial of Gold Mix, mix and pipette the sample (biomolecule in Reaction Buffer) directly onto the lyophilized material. Resuspend gently by withdrawing and re-dispensing the liquid once or twice using a pipette.

4.4 Place the cap back on the vial and leave it standing at room temperature (20-25˚C); 30 minutes incubation is recommended for proteins or antibodies, while 60 minutes is recommended for oligonucleotides; longer incubation times have no negative effect on the conjugate.

4.5 After incubating for 30 minutes (or more), add 5 µL (3 x 5 µg kit) or 50 µL (1 x 50 µg kit) kit) of Quencher Reagent (this is supplied as a lyophilized product and should be reconstituted in 100 µL of water; store surplus Quencher Reagent at -20°C after use). The conjugate can be used after 20 minutes. You now have 50 µL (3 x 5 µg kit) or 500 µL (1 x 50 µg kit) kit) of 20 OD conjugate.

4.6 Determining conjugate concentration:

The maximum absorbance for the 20 nm Gold maleimide is at 528 nm. To determine the effective concentration of the conjugate obtained we advise to measure the Abs_{max} of light using an UV-vis spectrophotometer after diluting your sample to an appropriate range for your piece of equipment.

*Δ **Note**: If you wish to exchange the gold conjugate into a specific buffer for your assay, centrifuge the conjugate in a*

microfuge at 9,000 x g for 20 minutes. Remove the supernatant and add your preferred buffer. The buffer should not contain thiols; all other common lab materials are acceptable after the conjugate has formed.

4.7 Antibody storage:

Storage at 4°C is recommended for any conjugate. A preservative may be desirable for long-term storage. The best conditions for any particular conjugate must be determined by experimentation.

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