Version 2c Last updated 14 August 2020

# ab269942 40nm Gold-Carboxyl Nanoparticles (40OD)

## A product of Expedeon, an Abcam company

Applicable to Expedeon product codes: 3400-0001, 3400-0005

View ab269942
40nm Gold-Carboxyl Nanoparticles (40OD):
www.abcam.com/ab269942
(use www.abcam.cn/ab269942 for China, or www.abcam.co.jp/ab269942 for Japan)

For the Covalent Conjugation of Antibodies or Proteins to Gold-Carboxyl Nanoparticles (40OD) using the water soluble carbodiimide N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC).

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

# Table of Contents



## 1. Overview

Carboxyl surface modification of gold nanoparticles is one of the most common functionalizations employed to covalently bind primary amine containing biomolecules (e.g. antibodies) using the water soluble carbodiimide N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC).

EDC is often used with NHS or sulfo-NHS to activate the carboxyl groups and then stabilize the intermediate ester formed which reacts with primary amines to form a stable amide bond.

40nm Gold-Carboxyl Nanoparticles (40OD) (ab269942) are optimized for single step EDC covalent coupling, without aggregation and crosslinking. Therefore, any EDC/NHS pre-activation and washing steps are unnecessary.

Another advantage over the traditional EDC/NHS coupling is the speed of the labeling process: the conjugate is ready to use in less than 35 minutes.

## 2. Materials Supplied and Storage

Store at +4°C immediately on receipt. Reagent can be stored for 1 year from receipt, if components have not been reconstituted.

40nm Gold-Carboxyl Nanoparticles (40OD) (ab269942) are shipped in 10mM EPPS pH 8.5. If you wish to exchange 40nm Gold-Carboxyl Nanoparticles (40OD) into a different buffer, centrifuge the particles in a microfuge at 2,500g for 10 minutes. Carefully remove the supernatant, gently tap the pellet and add your preferred buffer.

It is important to avoid substances that have a very high affinity for gold (e.g. thiols).

## 3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- EDC
- MES
- 1X TBS, 0.05% Tween 20
- BSA

## 4. Technical Considerations

### 4.1 Antibody Buffer Considerations:

There are a number of common buffer components that have a substantial negative effect on the conjugation efficiency. This decreases the amount of antibody/protein that will be coupled to each nanoparticle. To prevent this, we advise conjugating only from stock antibodies that are at least 1mg/ml, which allows some dilution of interfering substances, or that are already provided in a suitable buffer.

Please see the table below for recommended buffer conditions and components of the antibody to be labeled.

| Buffer Components | |
|---|---|
| pH | 6.5-8.5 |
| Amine free buffer * *(e.g. MES, MOPS, HEPES)* | Yes |
| Sugars | Yes |
| PBS* | No |
| Thiomersal | No |
| Thimerosal | No |
| Merthiolate | No |
| Sodium Azide | No |
| BSA | No |
| Gelatin | No |
| Tris | No |
| Glycine | No |
| Carboxylic acids *(e.g. EDTA, Citrate)* | No |
| Nucleophilic components *(Primary amines e.g. amino acids or ethanolamine and thiols e.g. mercaptoethanol or DTT)* | No |

***Δ Note:*** *Relatively weak buffers (e.g. 10 mM) are strongly preferred so that the pH conditions of the covalent reaction are not significantly altered upon addition of the antibody. Antibody concentrations >10mg/ml in PBS are acceptable.*

If your antibody buffer is not compatible with our kits, our Antibody Purification Kit range for Nanoparticles allows you to quickly and simply purify your antibody and is fully compatible with GOLD-Carboxyl Conjugation Kit.

## 5. Assay Procedure

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

**5.1** Prepare 100 mM and 10 mM MES buffers at pH 5.0.

**5.2** Dilute the antibody or protein to be conjugated down to 0.05-0.5 mg/mL in 10 mM MES pH 5.0. You may need to conjugate different amounts of antibody/protein to optimize your assay. For IgGs we recommend testing 0.1 mg/mL in the first instance, although slightly lower or higher concentrations can be explored to optimize performance in your particular application.

**5.3** Prepare a fresh solution of 50 mM EDC by adding 520 µL of ultrapure water to 5 mg of EDC and mix well.

**5.4** Dilute the EDC down to 1 mM by mixing 20 µL 50 mM EDC stock solution with 980 µL water. The EDC solution is not stable, use immediately.

**5.5** Add to 50 µL 40nm Gold-Carboxyl Nanoparticles (40OD):
- 10 µL 100mM MES pH 5.0 (step 1)
- 20 µL of diluted protein/antibody (step 2)

**5.6** Add 20 µL 1 mM EDC to the gold/protein and mix well (step 3 and 4).

**5.7** Incubate for 20 minutes at room temperature with mixing.

**5.8** After the incubation, add 1 mL of 1XTBS pH 8.0, 0.05% Tween20.

**5.9** Spin the gold conjugate down by centrifugation for 10 minutes at 2,500 x *g*.

**5.10** Carefully remove the supernatant without dislodging the pellet.

5.11 Tap the pellet and gently add 90 µL of 1XTBS pH 8.0, 0.05% Tween20, 0.5% BSA to obtain around 20 OD of conjugate. Mix well. For long term storage, avoid BSA in your buffer.

5.12 Conjugate Concentration Determination:

The maximum absorbance for the 40nm Gold-Carboxyl is at 530 nm. To determine the effective concentration of the conjugate obtained we advise to measure the absorbance of light at 530 nm using an UV-vis spectrophotometer after diluting your sample (e.g. if the conjugate is at 20 OD and is diluted 1:20 the Abs530 nm for a 1 cm light path is expected to be around 1 AU).

5.13 Storage of Conjugate:

For any new conjugate, initial storage at 4°C is recommended. Do not store the conjugate at -20□C.

The bond between the nanoparticle and antibody is covalent, via the surface, which means that the conjugates are very stable. Stability of individual antibodies on storage may vary and will need to be checked experimentally.

## Technical Support



**For all technical or commercial enquiries please go to:**

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)