



## **Carboxyl Gold Nanoparticles-PEG5K**

### **PRODUCT DATA SHEET**

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### **Description**

Gold nanoparticles are widely used nanomaterials and generally referred to as colloidal gold in biological research. Colloidal gold markers generally has a particle size between 10 and 100 nm, and will show different colors with the change of particle size. Gold nanoparticles have excellent biocompatibility, rich surface modification properties, and unique optical properties related to the surfactant, shape, size, and structure of the nanoparticles. According to their different characteristics, it can be applied to various fields of biomedicine, such as medical testing, medical imaging, drug delivery, etc.

Carboxylated gold nanoparticles are available with two different lengths of PEG surface spacers, i.e. 3000 Da and 5000 Da offering control of particle hydrodynamic size. These functionalized nanoparticles are ideal for conjugation of proteins using standard EDC/NHS coupling chemistry. Our carboxylated gold nanoparticles are available in 11 different sizes ranging from 5 ~ 100 nm, are more than 95% spherical and have uniform size distribution (CV < 12%).

Abvigen provides a variety of gold nanoparticles, gold nanorods, gold nanocages, gold nanostars, gold nanobipyramids, and other products, the product particle size is optional, the concentration can be customized, the surface can be modified with different groups, and can be appropriately selected according to the customer's use.

For custom sizes, formulations or bulk quantities please contact our customer service department.

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### **Characteristics**

Composition: Carboxyl Gold Nanoparticles-PEG5K

Shape: Spherical

Core diameter: 5 ~ 100 nm

Size dispersity: Coefficient of Variance (CV) < 12%

Polydispersity index (PDI): < 0.150

Size: 0.5 mL; 1 mL



Amount: OD = 50

Surface functional group: PEG5K-COOH

Absorbance ( $\lambda_{\text{max}}$ ): 510 ~ 570 nm

Nr of carboxyl groups on surface:  $\sim 1/\text{nm}^2$

Buffer: DI Water

Form: Suspension

Supplied in USP Grade H<sub>2</sub>O

### **Advantages**

Monodisperse

Well defined sizes from 5 nm to 100 nm

Precisely engineered surface with an optimized carboxyl group density for easy conjugation

Extensive range of surface functionalities designed for in vitro and in vivo applications

### **Application**

Ideal for development of gold conjugates for use in applications such as blotting, lateral flow assays, LSPR assays, light microscopy, and transmission electron microscopy (TEM) among others.

### **Storage**

This product should be stored at 4°C. **DO NOT FREEZE.** If stored as specified, Abvigen Carboxyl Gold Nanoparticles-PEG5K are stable for at least 12 months.

### **Handling**

When stored for a long period of time gold nanoparticles may sediment at the bottom of the vial, which is especially true for larger particle sizes. Prior to use, re-suspend the sedimented particles by swirling until a homogenous solution is obtained.

### **Note**

These products are for R&D use only, not for drug, household, or other uses.

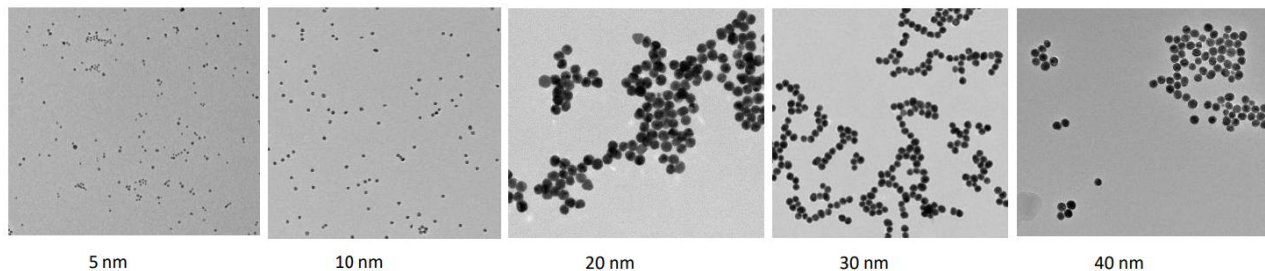
## NPS of Gold Nanoparticles, OD 50

Diameter	Peak SPR Wavelength	Optical density	Wt. conc	Size Dispersity %PDI	Particles/ml	Molarity mol/ml
5 nm	515-520 nm	OD 50	2.5 mg/ml	< 20%	1.98E+15	3.28E-09
10 nm	520 nm	OD 50	2.5 mg/ml	< 15%	2.47E+14	4.10E-10
20 nm	524 nm	OD 50	2.5 mg/ml	< 10%	3.09E+13	5.13E-11
30 nm	526 nm	OD 50	2.5 mg/ml	< 6%	9.15E+12	1.52E-11
40 nm	530 nm	OD 50	2.5 mg/ml	< 4%	3.86E+12	6.41E-12
50 nm	535 nm	OD 50	2.5 mg/ml	< 4%	1.98E+12	3.28E-12
60 nm	540 nm	OD 50	2.5 mg/ml	< 4%	1.14E+12	1.90E-12
70 nm	548 nm	OD 50	2.5 mg/ml	< 4%	7.21E+11	1.20E-12
80 nm	553 nm	OD 50	2.5 mg/ml	< 4%	4.83E+11	8.02E-13
90 nm	564 nm	OD 50	2.5 mg/ml	< 4%	3.39E+11	5.63E-13
100 nm	572 nm	OD 50	2.5 mg/ml	< 4%	2.47E+11	4.10E-13

## Gold Nanoparticles Centrifugation Parameters

Particle Size	Speed (g)	Time (min)
5 nm	100000	30
10 nm	17000	60 (~ 50% recovery)
20 nm	6500	30
30 nm	4500	30
40 nm	2500	30
50 nm	2000	30
60 nm	1125	30
80 nm	400	30
100 nm	400	30

## TEM of Abvigen gold nanoparticles of different size





## Covalent Conjugation to Carboxylated Gold Nanoparticles

Our Carboxyl Gold Nanoparticles rely on EDC/NHS chemistry for conjugation. EDC and NHS “activate” the carboxyl groups on the particle surface to form an intermediate that can subsequently react with primary amine groups on the specific protein or other ligand to be conjugated.

The following protocol provides general guidelines for coupling biomolecules to our Carboxyl Gold Nanoparticles, with conjugation of a standard IgG to our 20nm Carboxyl Gold Nanoparticles given as an example. For conjugation of other biomolecules, the optimal conjugation conditions may vary. To obtain maximum conjugation to the particle surface, the amount of protein for conjugation is about 1 to 10X excess that of its theoretical quantity needed for full coverage.

### Materials and Equipment Required

- Carboxyl Gold Nanoparticles
- Negative control: Methyl Gold Nanoparticles
- 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) (Sigma, Cat# E1769)
- N-hydroxysulfosuccinimide (Sulfo-NHS) (Sigma, Cat# 56485)
- Blocker: Bovine Serum Albumin (BSA) (Sigma, Cat# A3059)
- Activation buffer: 2-(N-morpholino)ethanesulfonic acid (MES) buffer (10 mM, pH 5.5)
- Coupling buffer: 1X Phosphate Buffered Saline (PBS)
- Washing buffer: 1X Phosphate Buffered Saline + 0.05% Tween 20 (PBST)
- UV-VIS Spectrophotometer
- Protein of interest to be conjugated.

**Note:** For effective conjugation, the purity of the protein needs to be considered. Any other molecules containing primary amines (e.g. TRIS) may compete with the protein to be conjugated and reduce the conjugation efficiency. The protein should also have enough accessible primary amine groups for conjugation. Lysine residues are the primary target sites for EDC/NHS conjugation. A higher number of lysine groups on the outer surface of the protein will probably lead to higher conjugation efficiency. For example, bovine serum albumin (BSA) has 30 to 35 lysine groups available on its surface for EDC conjugation. An IgG antibody molecule typically has about 90 lysine residues, and 30 are potentially useful for conjugation.

### Procedure

1. Prepare fresh EDC/NHS mix solution in 10 mM MES buffer (pH 5.5) at a concentration of 30 and 36 mg/mL, respectively.



Note: EDC/NHS rapidly hydrolyzes in aqueous solutions and should be prepared fresh just prior to conjugation.

2. Remove a 10  $\mu$ L aliquot of 20 nm carboxyl gold nanoparticles (supplied at OD 50 in water) from the stock vial and mix with 10  $\mu$ L of EDC/NHS mix solution as prepared in step 1.
3. Incubate for 30 min at room temperature.
4. Add 1 mL of PBST and vortex thoroughly\*\*
5. Spin down by centrifugation at 6,500 g for 30 min
6. Remove most of the supernatant.
7. Add 10  $\mu$ L of IgG (1 mg/mL in 1X PBS)\*\*\*
8. Sonicate in a water bath sonicator for 10 sec.
9. Incubate for 2 to 4 h at room temperature with mixing.
10. Add 1 mL of PBST and vortex thoroughly.
11. Spin down by centrifugation at 3,500 g for 30 min
12. Remove most of the supernatant
13. Add 50  $\mu$ L PBS with 1% BSA
14. Store at 4 degrees and ready to use

\*\* For smaller proteins, peptides, and amine-modified oligonucleotides or other ligands a one-step conjugation procedure may be employed, i.e. simultaneous activation and conjugation.

\*\*\* The concentration of protein may vary depending on the particle size and protein to be conjugated. In general, the amount of protein should be 1X to 10X excess of the amount of full surface coverage.

### Frequently Asked Questions

Q: what is the optimal conjugation pH for conjugation?

A: The EDC/NHS prefers an acidic environment for higher conjugation efficiency. However, conjugation can occur at pH between 4.5 to 7.4. In our protocol, we activate the carboxyl groups at pH 5.5 first to maximize the carboxyl activation. The excess EDC/NHS is then washed away to prevent protein crosslinking. At this step, the protein to be conjugated can be in buffers of pH from 4.5 to 7.4, depending on the protein.

Q: what is the optional conjugation time?

A: 2 to 4 h at room temperature is generally optimal for proteins. Based on the stability of the protein to be tested, a shorter or longer conjugation time should be tested. The conjugation efficiency of EDC



is usually low, so a conjugation time of at least 2-hour is common. We recommend testing different incubation times to find the most optimal.

Q: what other factors can influence conjugation results?

A: If the conjugation pH and conjugation time are within the optimal range, but there is no conjugation, it is necessary to make sure EDC/NHS is freshly prepared just before conjugation. EDC should always be stored at -20 degrees. Effective removal of excess EDC/NHS after activation is important to prevent them from crosslinking proteins. Also ensure that your protein solution is free of any primary amine containing contaminants such as e.g. TRIS.

### **Ordering Information**

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