CD Bioparticles

Carboxyl Gold Nanoparticles Conjugation Protocol

Please read this instruction manual carefully before using the product

Page Finder

1. Description

Carboxyl gold nanoparticles are available with two different lengths of PEG surface spacers, i.e. 300Da and 5000Da offering control of particle hydrodynamic size.
These functionalized nanoparticles are ideal for conjugation of proteins using standard EDC/NHS coupling chemistry, see page 2 for a recommended protocol. Our carboxylated gold nanoparticles are available in 12 different sizes ranging from 5 -100nm, are more than 95% spherical and have uniform size distribution (CV <12%).

2. Features

- Superior size distribution compared to the leading competitor; available from 5nm to 100nm.
- Precisely engineered surface with an optimized carboxyl group density for easy conjugation.

3. Applications

- 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.

4. Characteristics

Core diameter: 5 -100nm (Coefficient of Variance < 12%)
Polydispersity Index (PDI): < 0.150
Amount: OD=50
Absorbance (λmax): 510-570nm
Nr of carboxyl groups on surface: ~ 1/nm^2
Supplied in USP Grade H_2O

5. Storage

This product should be stored at 4°C. Do not freeze. If stored as specified, Carboxylated Gold Nanoparticles are stable for at least 12 months.

Diameter (nm)	Peak SPR Wavelength (nm)	NPS/ml	Wt. Conc. (mg/ml)	Size Dispersity (+/-nm)	Surface Area (nm2)	Surface/ Volume Ratio	Particle Mass (g)	Molar Mass (g/mol)	Molar Conc.
10	515-520	2.99E+14	3.04	<15%	3.14E+02	0.6	1.02E-17	6.11E+06	4.97E-07
15	520	8.20E+13	2.81	<12%	7.07E+02	0.4	3.43E-17	2.06E+07	1.36E-07
20	524	3.27E+13	2.66	<12%	1.26E+03	0.3	8.12E-17	4.89E+07	5.45E-08
30	526	8.95E+12	2.46	<12%	2.83E+03	0.2	2.74E-16	1.65E+08	1.49E-08
40	530	3.58E+12	2.33	<12%	5.03E+03	0.15	6.50E-16	3.91E+08	5.95E-09
50	535	1.76E+12	2.23	<10%	7.85E+03	0.12	1.27E-15	7.64E+08	2.92E-09
60	540	9.80E+11	2.15	<10%	1.13E+04	0.1	2.19E-15	1.32E+09	1.63E-09
70	548	6.00E+11	2.09	<10%	1.54E+04	0.086	3.48E-15	2.10E+09	9.95E-10
80	553	3.91E+11	2.03	<10%	2.01E+04	0.075	5.20E-15	3.13E+09	6.50E-10
90	564	2.69E+11	1.99	<8%	2.54E+04	0.067	7.40E-15	4.46E+09	4.46E-10
100	572	1.92E+11	1.95	<8%	3.14E+04	0.06	1.02E-14	6.11E+09	3.19E-10

6. 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 mount 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 10mM MES buffer (pH 5.5) at a concentration of 30 and 36 mg/mL, respectively.
2. Note: EDC/NHS rapidly hydrolyzes in aqueous solutions and should be prepared fresh just prior to conjugation.
3. Remove a 10 µL aliquot of 20 nm carboxyl gold nanoparticles (supplied at OD 50 in water) from the stock vial and mix with 10 µL of EDC/NHS mix solution as prepared in step 1.
4. Incubate for 30 min at room temperature
5. Add 1 mL of PBST and vortex thoroughly**
6. Spin down by centrifugation at 6,500 g for 30 min
7. Remove most of the supernatant
8. Add 10 µL of IgG (1 mg/mL in 1X PBS)***
9. Sonicate in a water bath sonicator for 10 sec
10. Incubate for 2 to 4 hours at room temperature with mixing
11. Add 1 mL of PBST and vortex thoroughly
12. Spin down by centrifugation at 3,500 g for 30 minutes
13. Remove most of the supernatant
14. Add 50 µL PBS with 1% BSA
15. 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. The total surface area of particles and the docking area should be estimated to calculate the optimal amount of protein, see table I.

7. Validation of Conjugation

We recommend using a straightforward immuno-dot blot protocol to confirm successful conjugation of your antibody. A recommended procedure is described below.

8. IgG Immuno-Dot Blot Assay

Materials Required

- Antigen or Antibody (1 mg/ml in 1X PBS)
- Antibody or Antigen Gold Conjugate
- Blocking Solution – 5% (w/v) Dry Milk in 1X PBS
- Gold Conjugate Dilution Buffer – 1% (w/v) Dry Milk in 1X PBS
- Wash Solution – 0.05% (w/v) Tween 20 in H_2O
- Nitrocellulose Membrane (Whatman, Cat# 10 402 594C)
- Optional: Mini Incubation Trays (Bio-Rad, Cat#170-3902)

Procedure

1. Prepare a serial dilution of your antigen or antibody in 1X PBS: 0.01, 0.05, and 0.1 µg/µL.
2. Spot 1 µL of the above solutions onto a nitrocellulose membrane strip and let air-dry for at least 30 minutes.
3. Transfer the membrane strips to a Mini Incubation Tray or a regular glass/plastic 2-mL vial.
4. Add 1.5 mL of blocking solution (make sure the solution covers the entire membrane).
5. Put the tray or vials on a rocking plate and incubate for 30 minutes at room temperature.
6. Dilute your antibody or antigen gold conjugate to a final optical density of 0.2-0.5 with 1% (w/v) dry milk in 1X PBS.
7. Remove blocking solution from the tray or vial with the membrane.
8. Add 1.5 mL of gold conjugate prepared as in step 6 to the tray or vial.
9. Incubate for 2 hours at room temperature. For increased sensitivity, incubation can be performed over night.
10. Remove the gold conjugate solution.
11. Add 1 mL of water containing 0.05% Tween 20 to wash the membrane.
12. Remove the water and repeat washing step twice.
13. Add 1 mL of silver enhancing reagents (prepare freshly before use according to instructions in kit).
14. Develop for 15 minutes and observe color change.

9. 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 hours 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.

Table 1. Suggested quantities of IgG needed for conjugation to Carboxyl Gold Nanoparticles of different sizes. The docking area of IgG is estimated to be 45 nm^2, with a molecular weight of 150 kDa. "N X full coverage amount" means the excess ratio between the incubation amount and the amount needed for full coverage of particle surface.

Carboxyl Gold Particles					**Human IgG**				
Size	Vol (mL)	Conc (OD)	Total Surface Area (nm2)	Number of IgG molecules for full coverage	Docking area of IgG (nm^2)	Conc (mg/mL)	Vol (mL)	Number of IgG molecules	N X full coverage amount
5	1	50	2.1E+17	4.8E+15	45	5	1	2.00E+16	4.2
10	1	50	9.4E+16	2.1E+15	45	3	1	1.20E+16	5.8
15	1	50	5.8E+16	1.3E+15	45	2	1	8.00E+15	6.2
20	1	50	4.1E+16	9.2E+14	45	1	1	4.00E+15	4.4
30	1	50	2.5E+16	5.6E+14	45	1	1	4.00E+15	7.1
40	1	50	1.8E+16	4.0E+14	45	0.5	1	2.00E+15	5
50	1	50	1.4E+16	3.1E+14	45	0.5	1	2.00E+15	6.5
60	1	50	1.1E+16	2.5E+14	45	0.5	1	2.00E+15	8.1
70	1	50	9.0E+15	2.0E+14	45	0.5	1	2.00E+15	10
80	1	50	7.8E+15	1.7E+14	45	0.5	1	2.00E+15	11.4
90	1	50	6.8E+15	1.5E+14	45	0.5	1	2.00E+15	13.2
100	1	50	6.0E+15	1.3E+14	45	0.5	1	2.00E+15	14.9