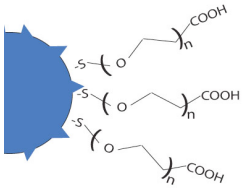
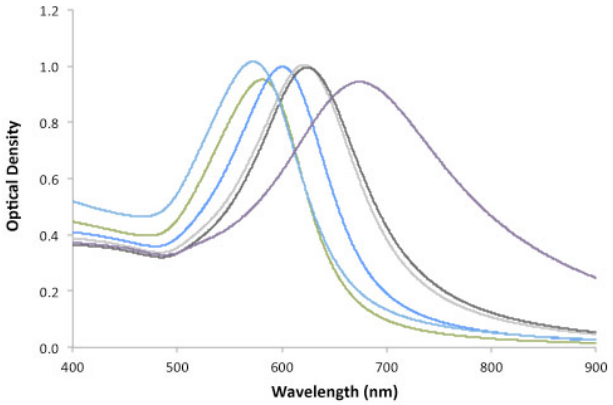
cytodiagnostics

PRODUCT DATA SHEET

Carboxylated Gold NanoUrchins





Description

Cytodiagnostics carboxylated gold nanourchins are available with two different lengths of PEG surface spacers, *i.e.* 3000Da and 5000Da offering control of particle hydrodynamic size.

These functionalized nanourchins are ideal for conjugation of proteins using standard EDC/NHS coupling chemistry, see page 2 for a recommended protocol.

Our carboxylated gold nanourchins are available in 6 different sizes ranging from 50 -100nm, and have uniform size distribution (CV <12%).

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

Features

• Superior size distribution compared to the leading competitor; available from 50nm to 100nm.
• Precisely engineered surface with an optimized carboxyl group density for easy conjugation.

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.

Characteristics

Core diameter: 50 -100nm (Coefficient of Variance < 10%)
Polydispersity Index (PDI): < 0.25
Amount: OD=50 (OD/ml = 50)
Absorbance (λmax): 580-680nm
Nr of carboxyl groups on surface: ~ 1/nm2
Supplied in USP Grade H_2O

Storage

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

Handling

When stored for a long period of time gold nanourchins 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.

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Diameter (nm)	Peak SPR Wavelength (nm)	NPS/ml	Wt. Conc. (mg/ml)	Size Dispersity (+/-nm)	Particle Volume (nm^3)	Surface Area (nm^2)	Surface/ Volume Ratio	Particle Mass (g)	Molar Mass (g/mol)	Molar Conc.
50	585	1.76E+12	2.23	<10%	6.54E+04	7.85E+03	0.12	1.27E-15	7.64E+08	2.92E-09
60	585	9.80E+11	2.15	<10%	1.13E+05	1.13E+04	0.1	2.19E-15	1.32E+09	1.63E-09
70	600	6.00E+11	2.09	<10%	1.80E+05	1.54E+04	0.086	3.48E-15	2.10E+09	9.95E-10
80	620	3.91E+11	2.03	<10%	2.68E+05	2.01E+04	0.075	5.20E-15	3.13E+09	6.50E-10
90	630	2.69E+11	1.99	<8%	3.82E+05	2.54E+04	0.067	7.40E-15	4.46E+09	4.46E-10
100	680	1.92E+11	1.95	<8%	5.24E+05	3.14E+04	0.06	1.02E-14	6.11E+09	3.19E-10

Covalent Conjugation to Cytodiagnostics Carboxylated Gold Nanourchins

Our Carboxyl Gold Nanourchins 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 Nanourchins, with conjugation of a standard IgG to our 50nm Carboxyl Gold Nanourchins 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 Nanourchins
- Negative control: Methyl Gold Nanourchins
- 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.

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

2. Remove a 10 μL aliquot of 20 nm carboxyl gold nanourchins (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.

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 μL of IgG (1 mg/mL in 1X PBS)***

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8. Sonicate in a water bath sonicator for 10 sec

9. Incubate for 2 to 4 hours 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 minutes

12. Remove most of the supernatant

13. Add 50 μL 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. The total surface area of particles and the docking area should be estimated to calculate the optimal amount of protein, see table I.*

Table 1. Suggested quantities of IgG needed for conjugation to Carboxyl Gold Nanourchins 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
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

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.

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)
- Optional: Silver Enhancer Kit for Membranes (Cytodiagnostics Cat# SR-01-02)

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.

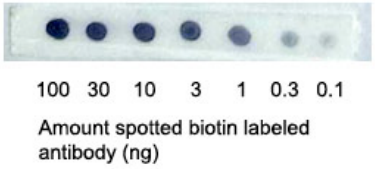

Figure 1. Detection of a biotinylated antibody spotted on a nitrocellulose membrane using streptavidin conjugated gold nanourchins.

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.

Related Products

- Conjugation Services – let us solve your problem!

Catalog Number	Description	Lambda max (nm)	Sizes
GUC3K-50- X*	50nm Carboxyl Gold Nanourchins (3000Da PEG)	585	0.5ml, 1.0ml (50 OD)
GUC3K-60- X*	60nm Carboxyl Gold Nanourchins (3000Da PEG)	585	0.5ml, 1.0ml (50 OD)
GUC3K-70- X*	70nm Carboxyl Gold Nanourchins (3000Da PEG)	600	0.5ml, 1.0ml (50 OD)
GUC3K-80- X*	80nm Carboxyl Gold Nanourchins (3000Da PEG)	620	0.5ml, 1.0ml (50 OD)
GUC3K-90- X*	90nm Carboxyl Gold Nanourchins (3000Da PEG)	630	0.5ml, 1.0ml (50 OD)
GUC3K-100- X*	100nm Carboxyl Gold Nanourchins (3000Da PEG)	680	0.5ml, 1.0ml (50 OD)
GUC5K-50- X*	50nm Carboxyl Gold Nanourchins (5000Da PEG)	585	0.5ml, 1.0ml (50 OD)
GUC5K-60- X*	60nm Carboxyl Gold Nanourchins (5000Da PEG)	585	0.5ml, 1.0ml (50 OD)
GUC5K-70- X*	70nm Carboxyl Gold Nanourchins (5000Da PEG)	600	0.5ml, 1.0ml (50 OD)
GUC5K-80- X*	80nm Carboxyl Gold Nanourchins (5000Da PEG)	620	0.5ml, 1.0ml (50 OD)
GUC5K-90- X*	90nm Carboxyl Gold Nanourchins (5000Da PEG)	630	0.5ml, 1.0ml (50 OD)
GUC5K-100- X*	100nm Carboxyl Gold Nanourchins (5000Da PEG)	680	0.5ml, 1.0ml (50 OD)

NOTE: X* is either -25 for 0.5ml or -50 for 1.0ml format.

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