

Conjugating Carboxyl-Polymer Spherical Gold Nanoparticles to Antibodies EDC Kit Part Number 21-PC-KIT1 Rev 1.2

Purpose

Conjugation, purification, and validation of conjugating Carboxyl-polymer SGNPs to Customer supplied IgG.

Introduction

This is a kit to instruct the conjugation of carboxyl-polymer spherical gold nanoparticles (SGNPs) to any primary amine using EDC chemistry. This kit requires the customer to supply an IgG as the conjugation target.

EDC is a carboxyl and amine-reactive zero-length crosslinker. EDC reacts with a carboxyl group first and forms an amine reactive *O*-acylisourea intermediate that quickly reacts with an amino group to form an amide bond and release of an isourea by-product (see the Additional Information Section). Failure to react with an amine will result in hydrolysis of the intermediate, regeneration of the carboxyl and release of an *N*-substituted urea. A side reaction is the formation of an *N*-acylurea, which is usually restricted to carboxyls located in hydrophobic regions of proteins.^{1,3}

Kit Includes

1. 21-PC-KIT1-1 Carboxyl-polymer SGNPs to IgG Conjugation Kit Instruction Manual
2. 21-PC-KIT1-2 C11 or C12-Carboxyl, Conjugated SGNPs in PBS, Carboxyl-polymer, xx nm, 50 OD mLs, where xx is the size of the nanoparticle delivered
3. 21-PC-KIT1-3 N-ethyl-N'-dimethylaminopropyl-carbodiimide (EDC), 10 mg in 1.5 ml microcentrifuge tube
4. 21-PC-KIT1-4 Nanopartz PBS based Buffer, pH=7.4, 5ml
5. 21-PC-KIT1-5 Customer supplied IgG, 100 μ l (1 mg/ml solution)
6. 21-PC-KIT1-6 1% PBS, 5mL
7. 21-PC-KIT1-7 Low binding 1.5 ml microcentrifuge tubes

Storage and Shelf Life

Refrigerate at 4°C. Shelf life is 14 days, limited by the IgG. Use only the EDC amount necessary – once dissolved in water its lifetime is less than one hour. Allow kit components to warm to room temperature prior to using.

Other required materials/instruments

1. Transfer pipettes for volumes ranging from 1 μ l to 2 ml

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2. Microcentrifuge with minimum rcf = 10,000 for 20 nm sizes and larger, for smaller sizes, please contact us for the rcf necessary
3. Vortex for microcentrifuge tubes
4. Sonicator

Other optional materials/instruments

1. Malvern Nano ZS DLS
2. UV VIS

Procedure

This procedure is for making 5 OD-mls of conjugated gold. To make more or less, adjust amounts proportionally. Make sure all components have been brought to room temperature.

1. Fill microcentrifuge tube (21-PC-KIT1-7) with 1.5 ml water (21-PC-KIT1-6). Add 100 µl of SGNPs solution (21-PC-KIT1-2). Vortex for 5 seconds. Save this as the control solution.
2. Add 100 µl of the SGNPs solution (21-PC-KIT1-2) to 400µl DI Water.
3. Add 10ug antibody, or for other protein, adjust for molecular weight so that an equal number of protein is similar.
4. Vortex for ~ 1 minute.
5. Add 1 ml DI water to 10 mg EDC (21-PC-KIT1-3) and immediately add 100 µL of this stock solution to the stirred solution from step 4.
6. Vortex for 15min.
7. Spin in microcentrifuge at a rate shown in Table 1 for 5 minutes or until a nice pellet is formed. Remove supernatant to less than 50 µl. Refill to 1.5 ml with Nanopartz PBS buffer (21-PC-KIT1-4). Repeat two more times. Sonicate between spins if necessary.
8. To ensure conjugation, on Malvern DLS measure size and charge for both control and the product from step 9. Note size increase and charge change.
9. Store at 4°C. Product good for 90 days. Resonicate if necessary.

Table 1. Spin speed

Diameter (nm)	rcf
5	80000
10	50000
20	10000
30	8000
40	6000
50	4000
60	3000
70	3000
80	2000
90	1000
100	600

Table 2. Size calculation from UV VIS

Diameter (nm)	Peak SPR Wavelength (nm)
5	514-520
10	514-520
15	514-520
20	514-520
25	514-520
30	524
35	526
40	527
45	529
50	531
55	533
60	536
65	539
70	542
75	545
80	549
85	553
90	558
95	563
100	569

Supplementary Information

EDC reacts with a carboxyl group first and forms an amine-reactive *O*-acylisourea intermediate that quickly reacts with an amino group to form an amide bond and release of an isourea by-product (Figure 1).

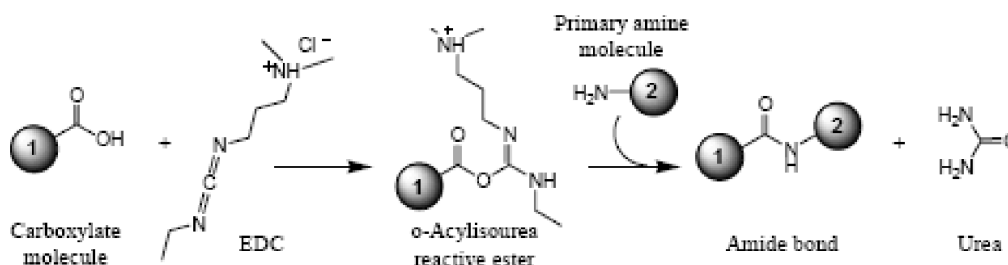


Figure 1 One-step EDC reaction with carboxyl and amine-containing molecules

References

1. Grabarek, Z. and Gergely, J. (1990). Zero-length crosslinking procedure with the use of active esters. *Anal Biochem* 185:131-5.
2. Staros, J.V., *et al.* (1986). Enhancement by *N*-hydroxysulfosuccinimide of water-soluble carbodiimide-mediated coupling reactions. *Anal Biochem* 156:220-2.
3. Timkovich, R. (1977). Detection of the stable addition of carbodiimide to proteins. *Anal Biochem* 79:135-43.

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