NANOPARTz™

The gold nanoparticle for nanotechnology

Conjugating Carboxyl-Polymer Spherical Gold Nanoparticles to Customer Antibodies Kit

21-PC-KIT2 rev 1.1

Product Profile

3/1/2009

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Purpose

Materials for conjugating Carboxyl-Polymer SGNPs to customer provided antibodies.

Introduction

This is a kit provides the material necessary for conjugation of carboxyl-Polymer spherical gold nanoparticles (SGNPs) to any primary amine using EDC chemistry.

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). The intermediate is unstable in aqueous solutions, and therefore, two step conjugation procedures require *N*-hydroxysuccinimide for stabilization.[1,2] 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 Applications

- Conjugate carboxyl to amine groups in peptides, and proteins.
- For DNA labeling through 5' phosphate groups, use the EDC chemistry to activate the phosphate group which then would conjugate to our amine terminated gold nanoparticles.

Kit Includes

1. 21-PC-KIT2-1 This Instruction Manual
2. 21-PC-KIT2-2 21-PC-xx-50, Conjugated SGNPs in MES, Carboxyl-Polymer, xx nm, 50 OD mLs, where xx is the size of the nanoparticle delivered
3. 21-PC-KIT2-3 N-ethyl-N'-dimethylaminopropyl-carbodiimide (EDC), 10 mg in 1.5 ml microcentrifuge tube
4. 21-PC-KIT2-4 Nanopartz PBS based Buffer, pH=7.4, 10 ml
5. 21-PC-KIT2-5 Customer supplied antibody or other with primary amine
6. 21-PC-KIT2-6 18 MEG DI H_20, 10 ml
7. 21-PC-KIT2-7 Low binding 1.5 ml microcentrifuge tubes

Storage and Shelf Life

Refrigerate at 4^oC. Shelf life is 14 days, limited by the customer supplied antibody or other with primary amine. 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 µl to 2 ml
2. Microcentrifuge with minimum rcf = 10,000 for 20 nm sizes and larger, for smaller sizes, please see below.
3. Vortex for microcentrifuge tubes
4. Sonicator

Other optional materials/instruments

1. Malvern Nano ZS DLS
2. UV VIS

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).

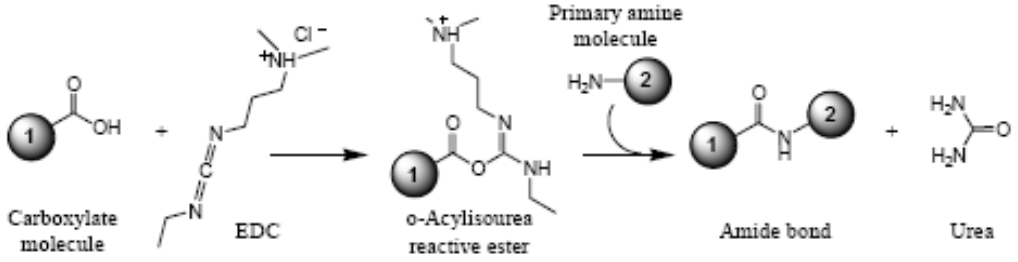

Figure 1 One-step EDC reaction with carboxyl and amine-containing molecules

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* 156:221-2.
3. Timkovich, R. (1977). Detection of the stable addition of carbodiimide to proteins. *Anal Biochem* 79:135-43.

There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

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