

NANO  **PARTz**TM

The gold nanoparticle for nanotechnology

Conjugating Carboxyl- Polymer Spherical Gold Nanoparticles to Primary Amine Kit Instruction manual

Part Number 21-PC-KIT3 Rev 1.1

Product Profile

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Purpose

Conjugation, purification, and validation of conjugating Carboxyl-polymer SGNPs to Customer primary amine. This kit makes 250 OD mLs of gold nanoparticles bound to primary amines.

Introduction

This is a kit to instruct the 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, proteins, and DNA labeling through 5' phosphate groups

Kit Includes

1. 21-PC-KIT3-1 Conjugating Carboxyl-Polymer Spherical Gold Nanoparticles to Primary Amine Kit Instruction manual
2. 21-PC-KIT3-2 Gold Nanoparticles sold separately
3. 21-PC-KIT3-3 N-ethyl-N'-dimethylaminopropyl-carbodiimide (EDC), 25 mg in 1.5 ml microcentrifuge tube
4. 21-PC-KIT3-4 Nanopartz PBS based Buffer, pH=7.4, 100 ml
5. 21-PC-KIT3-5 Customer supplied primary amine (1 mg/ml solution)
6. 21-PC-KIT3-6 18 MEG DI H₂O, 100 ml
7. 21-PC-KIT3-7 Low binding 1.5 ml microcentrifuge tubes (qty 10)

Storage and Shelf Life

Refrigerate at 4°C. Shelf life is 6 months. 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.

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Other required materials/instruments

1. Transfer pipettes for volumes ranging from 1 μ l to 2 ml
2. Microcentrifuge with minimum rcf = 10,000 for 20 nm sizes and larger, for smaller sizes, rcf's increase significantly (see table 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).

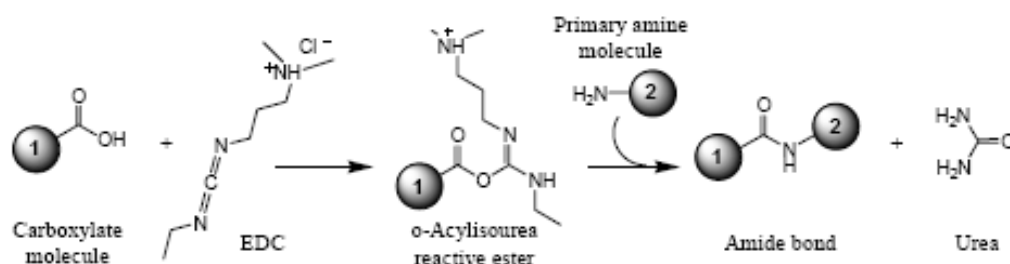


Figure 1 One-step EDC reaction with carboxyl and amine-containing molecules

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* 156:220-2.
3. Timkovich, R. (1977). Detection of the stable addition of carbodiimide to proteins. *Anal Biochem* 79:135-43.

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