



**NANO**●**PARTZ**<sup>™</sup>

The gold nanoparticle for nanotechnology

# Conjugating Carboxyl- Polymer Spherical Gold Nanoparticles to Customer Antibodies Kit

21-PC-KIT2 rev 1.1

Product Profile

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3/1/2009

## Purpose

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

## Introduction

This 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.<sup>1,2</sup> 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.<sup>1,3</sup>

## 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<sub>2</sub>O, 10 ml
7. 21-PC-KIT2-7 Low binding 1.5 ml microcentrifuge tubes

## Storage and Shelf Life

Refrigerate at 4°C. 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  $\mu$ l 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).

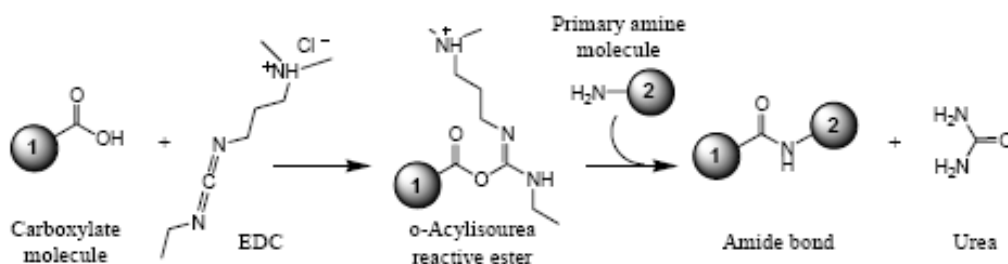


Figure 1 One-step EDC reaction with carboxyl and amine-containing molecules

## 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.

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