One Step Conjugations

Part Numbers:

CS, DS C,D-NHS

Introduction

The CS family of products include spheres in rods that have been encased in a polymer with N-hydroxysuccinimide (NHS) ester terminal groups. The proprietary polymer coating protects the gold nanoparticles from salt and variations in pH, and helps reduce non specific binding and aggregation in in vitro environments.

NHS esters react with primary amines at pH 7-9 to form amide bonds.

Important Product Information

Avoid buffers with primary amines (Tris, glycine) and sulfhydryls during conjugation because they will compete with the intended reaction. If necessary, dialyze samples into an appropriate buffer such as PBS.

Further, once hydrated the NHS groups will hydrolize within a few hours. Make sure to use the resuspended material immediately. Once functionalized, the product can be stored for months at 4C.

Materials

PBS. BupH™ Phosphate Buffered Saline Packs, part number 28372. https://www.lifetechnologies.com/order/catalog/product/28372. Potassium Free.

Tween. Fisher Scientific Tween 80, part number T164.

Nanopartz CS Kit.

Amine containing protein.

Sonicator: Branson 5510 Ultrasonic Cleaner/Water bath or a Cole Parmer 08890-01 42kHz 1-2 Amps

Centrifuge: Microcentrifuge capable of 12000 rcf.

Procedure

Generally a 100 to 500 fold molar excess of protein over the molarity of gold nanoparticles is sufficient to drive the reaction. Vortexing is recommended to drive the reaction.

A. Protocol

1. Add amine containing protein in PBS to NHS terminated gold nanoparticles. Use 100 micrograms in 1mL PBS of a 150kDa antibody to get 20 micrograms loaded onto the particles. For peptides, calculate the amount to be used by the following equation:

Wt of peptides = (150kDa/MW peptide) x 20ug.

If antibody or other amine containing target contains PBS or any other buffer, make sure the pH is between 7 and 9. Optimal results are found at pH 7-9. Immediately add this mixture to your gold nanoparticles.

2. Sonicate.

We typically use a Branson 5510 Ultrasonic Cleaner/Water bath or a Cole Parmer 08890-01 42kHz 1-2 Amps for 30 seconds. We make sure the water level is at a point where you can see visible sonication ripples in the water.

Typical sonication/shake times are 15 seconds to 1 minute.

3. Alternate sonicating and shaking of the microcentrifuge tube to resuspend gold nanoparticles and bound amine containing protein into solution.

4. To facilitate binding of the amine containing protein to the gold nanoparticles, vortex for 30 minutes at room temperature, and up to 30C for more efficient conjugations.

Purification is completed by centrifugation, removal of the supernatant, and resuspension using a diluted PBS mixture. These steps, steps 6, 7 and 8, are repeated three times.

6. Centrifugation speeds depend on centrifuge but in general, speeds from 8500 to 12000 rcf for 5 minutes are used for nanorods, 1500-15000 for spheres.

Product	rcf
10nm rods	12000
25nm rods	9000
Spheres 100nm	3500
Spheres 50nm	6000
Spheres 10nm	15000

7. Remove supernatant and refill with a 1% PBS 0.1% Tween solution. 1% PBS means dilute 1mL of standard PBS (with ~150mM NaCl) into 100mL of 18MEG DI water. Add 0.1mL of Tween 20 or 80. When refilling the last time, after the third centrifugation is

completed, refill with 100% PBS.

Conjugation efficiency may be estimated by electrophoretic separation and subsequent protein staining.