# NiNTA Functionalization Procedure

**Part Numbers:**

CS-NiNTA

## Introduction

The CS-NiNTA family of products include spheres in rods that have been encased in a polymer with Ni-NTA terminal groups. The proprietary polymer coating protects the gold nanoparticles from salt and variations in pH, and help reduce non specific binding and aggregation in in vitro environments.

Ni-NTA reacts with His protein tags at pH 7-9.

Important Product Information

## Procedure

Generally a 10 to 100 fold molar excess of protein over the molarity of NiNTA on the gold nanoparticles is sufficient to drive the reaction. Vortexing is recommended to drive the reaction.

A. Material Preparation

Conjugation Buffer: Sodium Borate.

Table top centrifuge.

Histag to be conjugated.

B. Protocol

1. Add his tag containing protein in SOBO to gold nanoparticles.

2. Alternate sonicating and shaking of the microcentrifuge tube to resuspend gold nanoparticles into solution.

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. Vortex for 30 minutes at room temperature, up to 30C for faster conjugations.

4. Purify by centrifugation. Centrifugation speeds depend on centrifuge but in general, speeds from 8500 to 12000 rcf for 10 minutes are used for nanorods, 1500-15000 for spheres.

| Product | rcf |
|---|---|
| 10nm rods | 11000 |
| 25nm rods | 8500 |
| Spheres 100nm | 1500 |
| Spheres 50nm | 4000 |
| Spheres 10nm | 12000 |

5. Repeat 3x.

Refill with a 1% SOBO 0.1% Tween solution. 1% SOBO means standard Sodium Borate diluted 100x. In the final centrifugation, refill with 100% SOBO.

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