CD Bioparticles

Absolute Mag™ Pyridyl Disulfide Magnetic Particles, 1 μm

Catalog Number: WNM-B108

Please read this instruction manual carefully before using the product

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1. Product Description

Pyridyl disulfides form disulfide connections with sulfhydryl groups over a wide pH range (the optimum is pH 4 to 5). A disulfide exchange happens during the reaction between the molecule's-SH group and the reagent's 2-pyridyldithiol group. The disulfide exchange can be conducted at physiological pH. However, the reaction rate is slower.

Because pyridyl dithiol compounds generate disulfide bonds with target sulfhydryls, conjugates created with these crosslinkers are cleavable using common disulfide reducing agents, such as dithiothreitol (DTT) or sample buffer for protein electrophores is (SDS-PAGE). Thus, pyridyl disulfide magnetic particles are useful alternatives to maleimide and haloacetyl reagents when it is necessary to reverse the sulfhydryl-conjugation step later in an experimental procedure (i.e., to exactly recover the original sulfhydryl-containing molecule). Pyridyl Disulfide Magnetic Particles are uniform magnetic particles coated with high-density thiol functional groups (2-pyridyl disulfide) on the surface (Fig.1). The particles can reversibly immobilize thiol-containing ligands under mild conditions. After affinity purification, reducing agents such as DTT or β-mercaptoethanol can cleave and separate the target molecule-ligand complex from the particles. Pyridyl Disulfide Magnetic Particles are most suitable for the conjugation of large proteins.

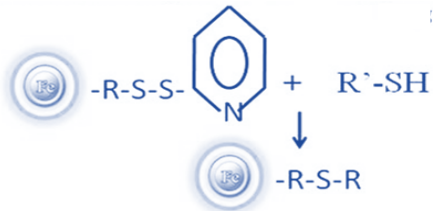

Fig.1 Structure of Pyridyl Disulfide Magnetic Particles

2. Specification

Composition: Magnetic particles are grafted with thiol groups.

Number of Particles: ~1.68 x 10^9 particles/mg

Stability: Short Term (<1 hour): pH 3-11; Long-Term: pH 4-10
Temperature: 4°C-140°C; Most organic solvents.

Magnetization: ~40-45 EMU/g

Type of Magnetization: Superparamagnetic

Formulation: Lyophilized Powder

Functional Group Density: ~240 µmol/g of Particles

Storage: Ship at room temperature. Store at -20°C.

3. Features and Advantages

- Pre-activated and ready-to-use.
- A cleavable built-in disulfide bond allows the ligand-target molecule. Complex separated from the particles.
- Specific isolation of cysteine proteins/peptides.
- Stable covalent bond with minimal ligand leakage.
- Produces reusable affinity matrix.
- Low nonspecific binding.
- Applications: Cell sorting, immunoprecipitation, purification for antibodies, proteins/peptides, DNA/RNA

4. Protocol

Note

The following protocol is an example for coupling protein and peptides to Pyridyl Disulfide Magnetic Particles. We strongly recommended titrating the quantity of particles used for each application. This protocol can be scaled up and down accordingly.

Workflow

The magnetic matrix works perfectly as affinity resin for a wide variety of affinity purification to refine thiol group-containing proteins or other molecules from the sample. After washing away unbound material, the thiol-containing substance is eluted by the addition of a reducing agent such as DTT or 2-mercaptoethanol.

Materials Required

- Coupling Buffer: 0.1 M sodium phosphate, pH 7.0, 5 mM EDTA
- L-Cysteine•HCl
- TCEP (tris(2-carboxyethyl) phosphine)
- Washing Buffer: 1 M NaCl, 0.05% NaN_3
- Magnetic rack (for manual operation)

Procedure

A. Ligand Preparation

Note:

- *Make sure that the protein/peptide to be conjugated has free (reduced) sulfhydryl. To ensure free sulfhydryl groups are available, treat the protein/peptide with a reducing agent such as DTT (dithiothreitol), TCEP (tris(2-*

carboxyethyl) phosphine), or 2-MEA (2-Mercaptoethylamine•HCl) followed by desalting or dialysis to remove the reducing agent.

- *Newly synthesized peptides may be directly used for coupling if used immediately after reconstitution.*
- For protein, treat protein with 5-10 mM TCEP solution for 30 minutes at room temperature, followed by dialysis or a desalting column. For IgG antibodies, 2-MEA is recommended due to its selective reduction of hinge-region disulfide bonds.
- If the sample contains reducing agents with free sulfhydryl (e.g., 2-mercaptoethanol, DTT, or TCEP), these agents must be entirely removed by dialysis or desalting.

1. Prepare 100 µL of protein solution (0.5-1 mg/mL) or peptide solution (200 µmol/mL) with coupling buffe.
2. If samples have already been suspended in another buffer, dilute samples with an equal volume of coupling buffer.

B. Magnetic Particles Preparation

1. Prepare 3% magnetic particles with Ethanol (30 mg/mL) and mix well. ***Note:*** *Store the unused particles in acetone solution at 4°C. It has been stabling for over a year.*
2. Transfer 100 µL (3 mg) magnetic particles to a centrifuge tube.
3. Place the tube on the magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the rack. Remove the tube from the rack and resuspend the particles with 1 mL coupling buffer by vortex for 30 seconds.
4. Repeat step 3 two times.
5. Remove the supernatant, and the washed particles are ready for coupling. ***Note:*** *Once rehydrated using the coupling buffer, use the particle as soon as possible due to the stability of the functional group.*

C. Coupling

1. Add the ligand to the washed magnetic particles and incubate at room temperature for 4-6 hours or overnight with continuous rotation. ***Note:*** *The user should optimize the incubation time.*
2. Wash the magnetic particles with 1 mL coupling buffer four times.
3. Block the excess active groups on the particles by suspending the particles in 1 mL Coupling Buffer containing 8 mg L-Cysteine•HCl and incubate 30-60 minutes at room temperature with gentle rotation.
4. Wash the particles with 1 mL washing buffer four times.
5. Resuspend the particles in PBS buffer containing 0.05% sodium azide and store them at 4°C.

D. General Affinity Purification Protocol

Note:

- *This protocol is a general affinity purification procedure. Designing a universal protocol for all protein purification is impossible because no two proteins are precisely alike. The user should determine the optimal working conditions for purifying the individual target protein to obtain the best results.*
- *Avoid reducing agents in binding and washing buffers.*
- *We strongly recommended titration to optimize the number of particles used for each application based on the amount of the target protein in the crude sample. Too many magnetic particles used will cause higher backgrounds, while too few particles used will cause lower yields. Each mg of magnetic particles typically binds to 10-20 µg of the target protein.*

1. Transfer the optimal amount of the particles to a centrifuge tube. Place the tube on the magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the rack.
2. Remove the tube and wash the particles with 5-bed volumes of PBS buffer by vortex for 30 seconds. Leave the tube at room temperature for 1-3 minutes. Place the tube on the magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the rack.
3. Repeat step 2 two times.
4. Add washed particles to the crude sample containing the target protein and incubate at room or desired temperature for 1-2 hours (Lower temperatures require longer incubation time). Note: Strongly recommended to perform a titration to optimize incubation time. More prolonged incubation may cause higher background.
5. Extensively wash the particles with 5-particles volumes of PBS buffer or 1 M NaCl until the absorbance of eluting at 280 nm approaches the background level (OD 280<0.05).

Note: *Adding a higher concentration of salts, nonionic detergent, and reducing agents may reduce the nonspecific background. For example, adding NaCl (up to 1-1.5 M), and 0.1-0.5% nonionic detergents such as Triton X100 or Tween20 to the washing buffer.*

6. Elute the target protein by appropriate methods such as low pH (2-4), high pH (10-12), high salt, high temperature, affinity elution, or boiling in SDS-PAGE sample buffer, or reducing agents.

7. Cleave the Disulfide Bond

Note: *Due to conformational variation from ligands to ligands, the user should determine the optimal working conditions such as reducing agent, pH, and temperature for cleaving the disulfide bond of individual ligands. The following is an example of cleaving conjugated GFP from the particles.*

1) Incubate the magnetic particles (30 mg/mL) in either 140 mM β-mercaptoethanol or 5 mM DTT (Dithiothreitol).

a. 100 mM Tris-HCl, pH 8.0, 50 mM EDTA, 140 mM β-mercaptoethanol for 2 hours to overnight at room temperature or 98°C for 5 minutes.

b. 100 mM Tris-HCl, pH 8.0, 50 mM EDTA, 5 mM DTT for 2 hours to overnight at room temperature or 98°C for 5 minutes.