CD Creative Diagnostics®

Protocol: Couple Ligands to Absolute Mag™ Tosyl Magnetic Particles

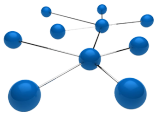
Cat# WHM-L081

Product Description

Absolute Mag™ Tosyl Magnetic Particles are solid-phase designed for biomagnetic separations. Any ligand (e.g. antibody, protein, peptide or glycoprotein) containing amino or sulfhydryl groups can be covalently coupled to the surface of the beads.
Absolute Mag™ Tosyl Magnetic Particles need to be coated with a ligand with affinity for e.g. the protein to be isolated. When added to a heterogeneous sample, the coated beads will bind to their target protein. After a short incubation, the sample is placed on a magnet for ligand separation.

Required Materials

- **Magnet**
- **Mixer allowing tilting and rotation of tubes**
- **Antibody or other selecting molecule.**
- **Phosphate Buffered Saline (PBS) pH 7.4.**
- **Coating Buffer**: 0.1 M sodium borate buffer pH 9.5:
  6.183 g H_3BO_3(MW 61.83). Dissolve in 800 mL distilled water. Adjust pH to 9.5 using 5 M NaOH and adjust volume to 1000 mL with distilled water.
  Note: The coating buffer is used for prewashing and coating of Absolute Mag™ Tosyl Magnetic Particles. Do not add any sugar, protein (apart from your ligand), i.e. to this buffer.
- **Stock Solution**: 3 M ammonium sulphate
  Preparation and handling shall be performed in a fume hood.
  39.6 g $(NH_4)_2SO_4$ (MW 132.1). Dissolve in 0.1 M sodium borate buffer (pH 9.5), control/adjust pH and adjust volume to 100 mL.
- **Blocking buffer**: PBS pH 7.4 with 0.5% with BSA and 0.05% Tween® 20:
  Blocking buffer is used for blocking of all pre-coated Absolute Mag™ Tosyl Magnetic Particles. Do not use this buffer or any buffer containing protein or amino-groups (glycine, Tris etc.) for pre-washing or coating of Absolute Mag™ Tosyl Magnetic Particles.
- **Washing/Storage Buffer**: PBS pH 7.4 with 0.1% with BSA and 0.05 Tween® 20:
  For storage purposes, add 0.02% sodium azide, as a preservative.
  Caution: Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.



- **Elution Buffers**:
  Any conventional method for protein elution can be used, e.g. 0.1 M citrate pH 3, 0.1 M glycine-HCl pH 2.5, or 0.1 M glycine-NaOH pH 10. All reagents used should be analytical grade.

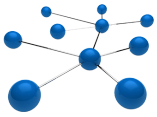
Protocols

To obtain optimal results, you should optimize buffer conditions, amount of ligand, bead and ligand concentration, blocking agents, and overall process economy. Antibody/protein to be coated directly onto the surface of the beads must be purified, since all proteins will bind to the bead surface. Sugars or stabilizers may disturb the binding and should be removed from the antibody/protein preparation.

Wash the Absolute Mag™ Tosyl Magnetic Particles:
This protocol is based on 50 mg (500 µL) beads.
1. Resuspend the beads in the vial (i.e. vortex for >30 sec, or tilt and rotate for 5 min).
2. Transfer 500 µL beads to a tube.
3. Add 1 mL Coating Buffer and resuspend.
4. Place the tube in a magnet for 1 min and discard the supernatant.
5. Remove the tube from the magnet and resuspend the washed beads in 100 µL Coating Buffer.

Suggested Coating Protocol
- This protocol is for coating of protein/antibody to 50 mg (originally 500 µL, but concentrated to 100 µL in the washing process) Absolute Mag™ Tosyl Magnetic Particles.
- Use ~2 mg protein/antibody (~40 µg antibody/mg beads). Calculate the ligand volume from the concentration (µg ligand/mL).
- Bead concentration during coating: 40 mg/mL giving coupling volume of 1250 µL.

1. To 100 µL washed beads add coating buffer (735 µL minus the calculated ligand volume) and mix properly.
2. Add the calculated amount ligand and mix properly.
3. Add the calculated amount of 3 M ammonium sulphate stock solution (415 µL).
4. Incubate for 16-24 hours at 37°C with slow tilt rotation (do not let the beads settle during the incubation period).
5. Place the tube on the magnet for 2 min, and remove the supernatant.
6. Add the same total volume (1250 µL) of Blocking Buffer and incubate at 37 °C over night.
7. Place the tube on the magnet for 2 min and remove the supernatant.
8. Add 1 mL Washing/Storage Buffer and resuspend.



9. Repeat steps 7–8 twice to give a total of 3 washes and resuspend the beads to the desired volume/ concentration.

Note:

For storage, add the desired preservative and store at 2°C to 8°C.
If the presence of BSA will interfere with your downstream application, this protein can be omitted from the buffer. Detergent may similarly be omitted.