CD Creative Diagnostics®

Protocol: Couple Ligands to Absolute Mag™ Tosyl Magnetic Particles

Cat# WHM-L075

Product Description

Absolute Mag™ Tosyl Magnetic Particles are ideal for protein isolation due to their size and surface chemistry. Any ligand (e.g. antibody, protein, peptide or glycoprotein) containing amino or sulfhydryl groups can be covalently coupled to the bead surface. The beads are also suitable for separating cells and bacteria, as a solid phase in rapid immunoassays, for molecular applications or screening of phage display libraries. The efficacy of immunomagnetic separation is critically dependant on the specificity and avidity of the antibody or other protein ligand applied. A ligand protein with affinity for the specific target protein (e.g. markers, receptors, enzymes) can be coupled to the surface of Absolute Mag™ Tosyl Magnetic Particles.

Required Materials

- **Magnet**
- **Mixer allowing tilting and rotation of tubes**
- **Primary antibody/ligand.**
- **Buffer A**: 0.1 M borate buffer pH 9.5
  6.18 g H_3BO_3 (MW 61.83). Dissolve in 800 mL distilled water. Adjust pH to 9.5 using 5M NaOH and adjust volume to 1 L with distilled.
- **Buffer B**: 0.1 M Na-phosphate buffer, pH 7.4
  2.62 g $NaH_2PO_4 \times H_2O$ (MW 137.99) and 14.42 g $Na_2HPO_4 \times 2\ H_2O$ (MW 177.99). Adjust volume to 1 L with distilled water.
- **Buffer C**: 3 M ammonium sulphate in Buffer A or B
  39.64 g $(NH_4)_2\ SO_4$ dissolved in Buffer A or B. Adjust pH with NaOH or HCl. Adjust up to 100 mL with Buffer A or B.
- **Buffer D**: PBS pH 7.4 with 0.5% (w/v) BSA
  Add 0.88 g NaCl (MW 58.4) and 0.5% (w/v) BSA to 80 mL 0.01 M sodium- phosphate pH 7.4. Mix thoroughly and adjust volume to 100 mL with 0.01 M sodium-phosphate pH 7.4.
- **Buffer E**: PBS pH 7.4 with 0.1% (w/v) BSA
  Add 0.88 g NaCl (MW 58.4) and 0.1% (w/v) BSA to 80 mL 0.01 M sodiumphosphate pH 7.4. Mix thoroughly and adjust volume to 100 mL with 0.01 M sodium-phosphate pH 7.4.

Buffer A, B and C are used for pre- washing and coupling of Absolute Mag™ Tosyl Magnetic Particles. Buffer A is the recommended buffer for coupling. For pH labile ligands, use Buffer B. Do not add any protein (apart from your specific protein ligand), sugar etc. to these buffers.
Buffer D and E are used for washing of all ligand-coupled beads. Buffer E can also be used for storage of ligand-coupled beads. Do not use these buffers or any buffer containing protein or aminogroups (e.g. glycine, Tris) for pre-washing or coupling to these beads. If BSA interferes with your downstream application, replace this with another protein (e.g. HSA) or a detergent (e.g. Tween® 20). Protein blocking is recommended as it reduces aggregation and non-specific binding. If a preservative is needed for the coupled beads, a final concentration of < 0.1% (w/v) sodium azide may be added to Buffer E.
Caution: Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

Protocols

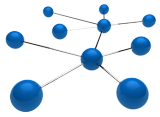
Wash the Absolute Mag™ Tosyl Magnetic Particles

1. Resuspend the beads in the vial (i.e. vortex for >30 sec, or tilt and rotate for 5 min).
2. Transfer the desired volume of beads to a tube.
3. Add the same volume of Buffer A or B, or at least 1 mL, 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 the same volume of Buffer A or B as the initial volume of beads (step 2).

Couple Ligands to the Absolute Mag™ Tosyl Magnetic Particles

- This protocol is based on 5 mg (~165 µL) Absolute Mag™ Tosyl Magnetic Particles. It is not recommended to couple < 5 mg beads at a time.
- Use 100 µg ligand/5 mg beads. The ligand volume (µL) is dependent on the ligand concentration (µg/uL) and must be calculated for each ligand, e.g. when coupling 5 mg beads, the Ab requirement is 100 µg. If the Ab concentration is 0.8 mg/mL you have to use 125 µL Ab (100 µg : 800 µg/mL = 0.125 mL).
- The optimal coupling concentration is ~40 mg beads/mL (step 4). It is decreased to 20 mg/mL when coupling the smallest amount (5 mg beads), to allow for a sufficient volume for efficient mixing.

1. Transfer 165 µL washed and resuspended beads to a new tube, place in a magnet for 1 min, and remove the supernatant.
2. Resuspend the beads in 100 µg ligand and add Buffer A (or B) to give a total volume of 150 µL. Mix thoroughly by vortex or pipetting.



3. Add 100 µL Buffer C and mix by vortexing or pipetting.
4. Incubate on a roller at 37°C for 12–18 hours.
5. Place the tube on a magnet for 2 min and remove the supernatant.
6. Remove the tube from the magnet and add 1 mL Buffer D, incubate at 37°C for 1 hour on a roller.
7. Place the tube on a magnet for 2 min and remove the supernatant.
8. Remove the tube from the magnet and add 1 mL Buffer E, vortex for 5–10 sec.
9. Place the tube on a magnet for 2 min and remove the supernatant.
10. Repeat steps 7–8 once.
11. Resuspend and dilute the beads in Buffer E to achieve your final desired bead concentration. For an example of 20 mg/mL, add 240 µL Buffer E.

Note:

If the ligand concentration is low, leave out the coupling buffer.

Optimal coupling concentration is 40 mg beads/mL, but can be reduced to 20 mg/mL if the coupling volume is low.