

His-tagged Protein Purification Resin

Catalog Number: IP999

Specifications and Use

- Resin** ♦ Agarose beads (60-160 μm) covalently linked to a murine monoclonal antibody specific for polyhistidine-tagged peptides.
- Quantity** ♦ 2 mL of 50% suspension in 20% ethanol resulting in 1 mL of settled resin.
♦ Centrifuge the vial before opening to recover entire contents of the vial. Due to possible sublimation during storage, the buffer volume may decrease over time. In this case, bring the volume up to 2 mL with 20% ethanol since the amount of the resin should remain the same.
- Applications** ♦ The resin is designed for the immunopurification of His-tagged proteins in a single step. The resin can also be used in small quantities to immunoprecipitate His-tagged proteins.
- Capacity** ♦ Each mL of packed resin can bind more than 0.5 mg of His-tagged proteins. Capacity is affected by many variables such as properties of the target and contaminating proteins and binding and washing conditions used.
- Recommended Conditions** ♦ The flow rate may be variable. For binding and elution steps, 1 mL/min. is recommended. For equilibration and washing steps, up to 5 mL/min. can be used.
♦ The resin can be equilibrated with a variety of buffers that are used for antigen and antibody binding. Typically, conditioned media from mammalian systems can be loaded directly onto this resin (sterile-filtering of such samples is recommended to prevent fouling of column by cell debris).
♦ Washing of non-specifically bound proteins can be done with equilibration buffer or a neutral buffer containing up to 1 M NaCl, 0.5% Triton X-100 and 0.25% Tween 20 (use of detergents is strongly recommended for best results).
♦ Elution of His-tagged proteins is achieved using an acidic buffer (e.g. 100 mM glycine, pH 2.5). To minimize the exposure of the purified protein to the low pH, it is recommended to add a neutralization buffer (e.g. 2 M Tris HCl, pH 7.5) to tubes for fraction collection of eluted samples.
♦ Re-equilibrate the resin in a neutral buffer immediately after elution to ensure stability of the resin.
- Regeneration** ♦ The resin can be used several times without loss of capacity. If performance does decrease, the resin should be washed with high salt (1 - 2 M NaCl). Low concentrations of urea (up to 2 M) can also be used to regenerate resin.
- Storage** ♦ The resin is shipped in 20% ethanol and should be stored at 2 - 8° C until use. The resin can be stored for 1 year at 2 - 8° in 20% ethanol.
♦ After use, resin should be regenerated and stored in 20% ethanol or a neutral buffer containing a bactericide such as sodium azide or Proclin 300.