Product Information

Product Name:	PabPur SulfoLink Beads
Product Code:	SA018005/SA018025
Size(s):	5ml & 25ml

Product Description:

PabPur SulfoLink Beads is an efficient affinity chromatography medium designed for the covalent immobilization of sulfhydryl-containing protein ligands to the agarose support matrix in affinity purification procedures. PabPur SulfoLink Beads is composed of the coupling of 4% agarose beads to iodoacetyl groups containing free (reduced) sulfhydryl groups. Free sulfhydryl groups reacts specifically with the iodoacetyl groups on the PabPur SulfoLink Beads medium to form a stable thioether linkage. PabPur SulfoLink Beads is ideally suited for the conjugation of sulfhydryl-containing peptide in polyclonal antibody purification, while obtaining high valences of antibody.

Product Specification:

Matrix:	4% agarose beads
Ligand:	Iodoacetic acid
Binding Capacity:	>3mg IgG/ml medium
Particle Size:	45 - 165μm
Maximum Pressure:	1bar (0.1MPa)
pH Stability:	5 - 10
Storage Conditions	2 – 8°C, 20% ethanol

Test Items	Specifications
Appearance	White to off-white slurry
Volume	50% suspension
Binding Capacity	>3mg IgG/ml medium

Operation Protocol

1. **Buffer Preparation**
   Water and chemicals of high purity should be used. It is recommended to filter all buffers through a 0.22μm or 0.45μm filter before usage.

Coupling Buffer: 50mM Tris-HCl, 5mM EDTA-Na, pH8.5
Blocking Buffer: 50mM Tris-HCl, 5mM EDTA-Na, 50mM L-Cysteine, pH8.5
Binding Buffer: 20mM sodium phosphate, pH8.0
Elution Buffer: 100mM glycine, pH2.5 – 3.0
Storage Buffer: 20mM sodium phosphate, 20% ethanol, pH8.0
Neutralising Buffer: 1M Tris-HCl, pH8.5

2. Sample Preparation

The sample should be filtered through a 0.22μm or 0.45μm filter before usage
The immobilized peptide/protein must have free (reduced) sulfhydryls.

i. If there is an absence of sulfhydryl groups for coupling, the disulphide bonds have to be cleaved with a reducing agent, such as TCEP (Tris(2-carboxyethyl)phosphine)
ii. If a sulfhydryl-containing reducing agent is used, desalting or dialysis must be performed to eliminate the reducing agent before immobilization

3. Coupling of Peptide/Protein to PabPur SulfoLink Beads

3.1. Resuspend and pack the PabPur SulfoLink Beads into the desired affinity column system. Allow the resin to settle down and the buffer to drain out from the column
3.2. To equilibrate the resin, add 3 column volumes of Coupling Buffer into the column and drain the buffer. Repeat this step twice
3.3. Apply the prepared sulfhydryl-containing sample (1 mg/mL, dissolved in Coupling Buffer) to the column. Shake the sample for 30 minutes at 28°C
3.4. Allow the buffer to drain from the column
3.5. Wash the medium with 3 column volumes of Coupling Buffer
3.6. An equal column volume of the Blocking Buffer is added into the column. Shake the sample for 30 minutes at 28°C. Drain the buffer
3.7. The column is prepared for purification

Note: If the column is not immediately used, wash the medium with 3 column volumes of Binding Buffer. Store the column with Storage Buffer 2 - 8°C

4. Column Purification Protocol

4.1. Resuspend the medium coupled with sample completely. Fill a new column with Binding Buffer before transferring the resuspended sample into a new column
4.2. Allow the resin to settle down and allow the buffer to drain from the column. Wash the column with 5 - 10 column volumes of Binding Buffer
4.3. Apply the sample into the column and drain the flow-through. Collect the flow-through to measure the binding efficiency of the resin through SDS-PAGE analysis
4.4. Wash the column with 10 column volumes of Binding Buffer, until the absorbance of the effluent is stabilised at 280nm
4.5. Elute the column with 5 column volumes of Elution Buffer. Collect the eluate and immediately neutralise to pH 7.4 with Neutralization Buffer (1/10 volume of the total eluate)

5. Results Analysis

The eluted fractions can be analysed using UV absorbance, SDS-PAGE and western blot

Storage

For long-term storage, store the PabPur SulfoLink Beads in 20% ethanol at 2 - 8°C

Troubleshooting Guide

Problems	Possible Causes	Solutions
The coupling efficiency is too low	There is a lack of free sulfhydryl groups and they are oxidized	To prevent the reformation of disulphide bonds, add DTT or TCEP before proceeding to the coupling procedure immediately after desalting
The flow rate of the column is very low	Tiny air bubbles from the buffer or particles from the sample block the gel pores	De-gas the buffers and samples. The column should not be allowed to dry
Peptide/protein precipitation in the Coupling Buffer	The protein/peptide is insoluble in Coupling Buffer	Dissolve the sample in either ≤ 30% DMSO, DMF or 6M guanidine-HCl in the Coupling Buffer
The eluted has a low purity	The column was not thoroughly washed	Increase the volume of the Binding/Wash Buffer