



Pabur SulfoLink Beads

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1. Product Description

PabPur SulfoLink Beads enable simple and efficient covalent immobilization of sulfhydryl containing peptides ,protein and other ligands to a medium for use in affinity purification procedures. The medium reacts specifically with free sulfhydryls to form a stable thioether linkage.**Pabpur Sulfolink Beads** is an indispensable purification medium for polyclonal antibody production. The characteristics of **PabPur SulfoLink Beads** are summarized in Table 1 .

Table 1. Characteristics of **PabPur SulfoLink Beads**

Item	Description
Matrix	4% agarose
Ligand	Iodoacetic acid
Capacity	> 3mg IgG/ml medium
Particle	45-165 μ m
Maxi pressure	0.1 MPa, 1 bar
pH stability	5-10
Storage buffer	1 M NaCl
Storage	2 $^{\circ}$ C-8 $^{\circ}$ C

2. Coupling and Purification Procedure

2.1 Buffer Preparation

Water and chemicals used for buffer preparation should be high purity. It is recommended to filter the buffers by passing them through a 0.22 μ m or 0.45 μ m filter before use.

Coupling Buffer: 50 mM Tris-HCl, 5 mM EDTA-Na, pH 8.5

Blocking Buffer: 50 mM Tris-HCl, 5 mM EDTA-Na, 50 mM L-Cysteine, pH 8.5

Storage Buffer: 20 mM sodium phosphate, 20% ethanol, pH 8.0

Binding Buffer: 20 mM sodium phosphate, pH 8.0

Elution Buffer: 100 mM glycine, pH 2.5-3.0

Neutralizing Buffer: 1 M Tris-HCl, pH 8.5

2.2 Sample Preparation

It is recommended to filter the sample solution by passing them through a 0.22 μ m or 0.45 μ m filter before use.

2.3 Couple to PabPur SulfoLink Beads

- 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 from the column.
- 2) Add 3 column volumes Coupling Buffer onto the column to equilibrate the resin and drain the buffer. Repeat the above test for 2 times.
- 3) Apply the sample(1mg/ml, dissolved in Coupling Buffer), shaking 30min at 28 $^{\circ}$ C. Allow the buffer to drain from the column. Wash the medium with 3 column volumes Coupling Buffer.
- 4) Add equal column volume Blocking Buffer onto the column, shaking 30min at 28 $^{\circ}$ C. Drain the buffer.
- 5) If you test it immediately, it can be directly to 2.4. Otherwise, Wash the medium with 3 column volumes Binding Buffer. Store with storage buffer at 2 $^{\circ}$ C-8 $^{\circ}$ C.





2.4 Column Purification

- 1) Resuspend completely the medium coupling with sample and transfer it to a new column, in which Binding Buffer was added in advance.
- 2) Allow the resin to settle down and the buffer to drain from the column. Wash the column with 5-10 column volumes of Binding buffer.
- 3) Apply the sample onto the column and drain the flow-through. Collect the flow-through for measuring the binding efficiency to the resin, i.e. by SDS-PAGE.
- 4) Wash the column with 10 column volumes of Binding buffer until the absorbance of the effluent at 280 nm is stable.
- 5) Elute with 5 column volumes Elution Buffer. Collect the eluate and immediately neutralize to pH 7.4 with Neutralization Buffer (1/10 volume of total eluate).

2.5 Analysis

Identify the fractions containing the target protein. Use UV absorbance, SDS-PAGE, or western blot.

3. Troubleshooting

Problem	Probable cause	Solution
The flow rate of the column is very low.	Tiny air bubbles from buffer or particles from sample block the gel pores.	De-gas buffers and samples. Do not allow the column to dry.
Protein/peptide precipitates in coupling Buffer	Protein/peptide is not soluble in Coupling Buffer.	Dissolve sample in $\leq 30\%$ DMSO or DMF or 6M guanidine-HCl in Coupling Buffer.
Low coupling efficiency	Sulfhydryl groups are not free, they are oxidized.	Add DTT or TCEP and proceed immediately to coupling procedure after desalting to prevent reformation of disulfide bonds.
The purity of elution fraction is low	The column was not washed thoroughly.	Increase the volume of Binding/Wash Buffer

4. Related Products

Product	Cat. No.	Size
PabPur SulfoLink Beads 4FF	SA069005	5 ml
	SA069025	25 ml
	SA069100	100 ml
	SA069250	250 ml
PabPur SulfoLink Beads	SA018005	5 ml
	SA018025	25 ml
	SA018100	100 ml
	SA018250	250 ml

