LT BIOTECH LTD,
Mokslininkų g. 6A,
LT-08412 Vilnius,
Lithuania
www.ltbiotech.lt

Phenyl Persefose HP

1. Basic product information

Phenyl Persefose HP is a hydrophobic interaction chromatography resin that uses the difference in the nature and amount of hydrophobicity of different molecules under specific conditions to separate them. This series of chromatographic resin can be used effectively for the separation and purification of various biological molecules such as recombinant proteins, antibodies, viruses and vaccines.
Phenyl Persefose HP has excellent scale-up performance:
(1) The highly cross-linked agarose matrix has excellent rigidity, so it can achieve high process flow rate under low back pressure and improve process efficiency.
(2) It has a fine particle size design to improve resolution.
(3) The hydrophilic base frame minimises the influence of the ligand on the hydrophobicity.
(4) Through chemical modification, it has excellent chemical compatibility and is resistant to cleaning-in-place (CIP) such as sodium hydroxide.

2. Chromatography resin parameters

Resin type	Hydrophobic interaction
Functional group	R-O-CH_2-CH(OH)-CH_2-O-C_6H_5
Matrix	Highly cross-linked agarose
Median particle size	34 µm
Ligand density	~25 µmol phenyl/ml
Dynamic binding capacity*	>45 mg α-chymotrypsinogen/ml
Recommended flow rate	90–150 cm/h
Maximum flow rate	200 cm/h
Maximum working pressure	3 bar
Working temperature	4–30°C

** Measuring conditions of dynamic binding capacity: column height, 10 cm; test flow rate, 150 cm/h; test buffer, 0.1M potassium phosphate, 2M ammonium sulfate solution, pH7.0; test sample, 2 mg/ml α-chymotrypsinogen, when α-chymotrypsinogen breakthrough reaches 10% of starting concentration.*

3. Chemical resistance

pH stability*	2–14
Chemical stability	All commonly used aqueous buffers, 3M ammonium sulfate, 30% isopropanol**, 75% ethanol**, 10% ethylene glycol**, 1M NaOH, 1M acetic acid, 6M guanidine hydrochloride, 8M urea
Avoid	Oxidising agents

** The physical and chemical properties and functions of the chromatographic resin did not change significantly after being placed in an environment of 40°C and pH 2–14 for 7 days.*

***v/v, volume ratio*

LT BIOTECH LTD,
Mokslininkų g. 6A,
LT-08412 Vilnius,
Lithuania
www.ltbiotech.lt

4. Method of use

4.1 Chromatographic conditions

(1) A buffer salt whose buffer group does not interact with the chromatographic resin should be selected. If the binding and elution mode is used, the equilibration buffer should be a high-salt buffer (such as a buffer containing 1.5-2 M ammonium sulfate) to facilitate the binding of the target molecule. The stability of the sample in the buffer should be considered; the elution buffer is usually a buffer with small content of salt. If the flow-through mode is used, the equilibration buffer should adopt conditions that are conducive to the binding of impurities. After the target molecule has completely flowed through, it should be washed directly with low-concentration salt.
(2) Flow rate: generally, choose a linear flow rate of 90–150 cm/h according to the column bed height.
(3) Sample pretreatment: to prevent the sample from clogging the column, the sample needs to be filtered with a 0.45 μm microporous membrane before loading it. It is recommended that the pH and conductivity of the sample are adjusted to be consistent with the equilibration buffer (dilution, ultrafiltration can be used and desalting to adjust the pH and conductivity of the sample).
‘

4.2 Chromatography steps

(1) Equilibration: use equilibration buffer to fully equilibrate the chromatography column until the pH and conductivity are stable and basically consistent with the equilibration buffer. This step usually requires 3–5 column bed volumes (CV).
(2) Sample loading*: according to the binding capacity measured in the small test, determine the sample loading volume and loading amount of the sample on Phenyl Persefose HP.
(3) Impurity washing*: use equilibration buffer or other suitable buffer to wash the chromatography column until the UV stabilises and returns to the baseline.
(4) Elution*: elution is achieved by decreasing the concentration of salt ions. The concentration of salt ions in the elution buffer can be gradually decreased through a linear gradient or a step gradient to elute molecules with different binding strengths. pH gradient elution or mixed elution can also be used.
(5) Regeneration: rinse the column with a buffer containing low salt.
(6) Re-equilibration: re-equilibrate the column with equilibration buffer.
* Note: If the flow-through mode is used, the ‘sample loading’ step should be set to collect; the ‘washing’ step should ensure that the target molecules have completely flowed through, then the collection can be stopped; the ‘elution’ step should directly use low-salt buffer – impurities can be washed away.

5. Cleaning and regeneration

Contaminants (such as lipids, endotoxins and proteins) accumulate on the column as the number of uses of the chromatography resin increases. Determine the frequency of CIP according to the degree of contamination of the chromatography resin (if the contamination is considerable, CIP is recommended after each use to ensure repeatability of results and to prolong the working life of the chromatography resin). For different types of impurities and contaminants, the recommended cleaning conditions are as follows:
• Removal of strongly binding proteins: wash with 5 CV of ultrapure or pure water.
• Removal of strongly hydrophobic proteins and precipitated proteins: first wash with 5 CV of 1M NaOH solution, then wash the lye with 5–10 CV of ultrapure or pure water.

LT BIOTECH LTD,
Mokslininkų g. 6A,
LT-08412 Vilnius,
Lithuania
www.ltbiotech.lt

• Removal of lipoproteins and lipids: first wash with 5 CV of 70% ethanol or 30% isopropanol, then rinse with 5–10 CV of ultrapure or pure water.
Note: 70% ethanol or 30% isopropanol should be degassed before use; the flow rate should be 30–60 cm/h during CIP. Reverse cleaning should be used when the clogging is severe.

To reduce the microbial load, it is recommended that 0.5~1M NaOH solution is used to treat the chromatographic resin. Treatment time is 15~30 minutes.

6. Storage

Keep the unopened chromatography resin in the original container and store at 4~30°C in a well-ventilated, dry and clean place. Do not freeze. Wash the used column with 2–3 CV of 20% ethanol solution and store at 2~8°C.

7. Destruction and recycling

Since chromatography resin is difficult to degrade in nature, it is recommended that the waste chromatography resin is incinerated to protect the environment. For chromatography resin that has been in contact with biologically active samples such as viruses and blood, follow the local biosafety requirements before destroying or disposing of it.

8. Packing method

Detailed information on resin packaging is available on request. Please contact your local distributor.

9. Ordering information

Product name: Phenyl Persefose HP

Product Cat. No	Package
730-00025	25 ml
730-00100	100 ml
730-00500	500 ml
730-01000	1 L
730-05000	5 L
730-10000	10 L
730-20000	20 L