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Sepapure Glutathione FPLC column

Short guide

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Note: For your own safety, read the instructions and observe the warnings and safety information on the device and in the instructions. Keep the instructions for future reference.

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1. Specifications

Definition Sepapure Glutathione columns are designed for the purification of Glutathione S-Transferase (GST) tagged proteins. The columns may also be used for the purification of other glutathione-binding proteins and glutathione S-transferases. The columns are designed to be used with low-pressure FPLC-type automated purification systems and operated below 3 bar (0.3 MPa, 2.96 atm).

Hardware specifications

Column housing	Polypropylene
Frits	Polyethylene (nominal 20 µm porosity)
Fittings	10/32 UNF
Bed volume	1 and 5 ml

1.1 Resin specifications

Resin name	Sepapure Glutathione FF
Base matrix material	4% cross-linked, beaded agarose
Mean bead diameter	100 µm
Ligand	Glutathione
Static protein binding capacity	> 10 mg/ml (dependent on protein)
Flow rate	> 300 cm/h
pH stability	3 – 12

Note: Before use, inspect the column for damage. If any damage is observed, do not use the column.

Note: Flow rates shown in this manual are for guidance only. Always ensure that system pressure is below the maximum for the column and resin.

1.2 Buffers

Sepapure columns are designed to be used with most aqueous phase chromatography buffers. A suggested buffer system is shown below, although other buffers may be used.

Equilibration buffer	PBS, pH 7.4
Wash buffer	PBS, pH 7.4
Elution buffer	50 mM Tris-HCl containing 10 mM reduced glutathione, pH 8.0

2. Preparing the column

Sepapure Glutathione columns are supplied with 20% ethanol as the storage buffer. This must be removed prior to purification.

Process 1. Remove the end-plugs and connect the column to the control system, taking care to avoid introduction of air into the system.

Note: Do not over-tighten fittings as this can strip the screw connections and lead to column leakage.

2. Flush the column with 3 to 5 Column Volumes (CVs) of binding buffer at a flow rate of 1 to 2 CV/min to remove the storage buffer.
3. Equilibrate the column with 3 to 10 CVs of binding buffer at 1 CV/min to ensure that pH, conductivity and UV_{280} signals are stable.

Result The column is now ready for use.

3. Sample preparation

Sepapure Glutathione columns should only be used with clarified (particle-free) samples. If the protein is expressed in inclusion bodies, it can be released through the addition of denaturing reagents such as guanidine hydrochloride or urea. To reduce the effects of sample viscosity when performing this step, it may be necessary to treat the sample with DNAse before application to the column. The sample should be adjusted to approximately pH 7.4 before purification.

4. Protein purification

Process 1. Load the sample at a flow rate of approximately 0.5 to 1 CV/min. Some glutathione - protein binding kinetics and sample viscosities will require the flow rate to be adjusted.

Note: To avoid overloading the column, the amount of sample that is applied to the column should not exceed the binding capacity of the solid phase to the target molecule.

2. Once the sample has been loaded, wash the column with binding buffer until the UV_{280} trace reaches baseline.
3. If a wash buffer is used, then also wash the column with 3 to 5 CVs of this reagent.

4. Elute the protein using a selected elution buffer. A stepwise or linear gradient may be used to determine the precise elution point of the target antibody. Initially, all elution fractions should be collected for further analysis. Buffer exchange and/or desalting might be required following elution and we recommend Sepapure Desalting Columns (010X460SPZ and 020X460SPZ) for this purpose.

5. Column regeneration

Process

To remove contaminants (lipids, non-specifically bound proteins, etc.) from the column following elution, wash with a further 3 to 5 CVs of elution buffer, followed by 3 to 5 CVs of equilibration buffer.

Ionically bound contaminants can also be removed using 2M NaCl.

Contaminants of a more hydrophobic nature may be removed by using detergents (0.1 – 2% non-ionic), 70% ethanol, 30% isopropanol under either acidic (eg.: acetic or phosphoric acids) or basic conditions. Combinations of the aforementioned reagents may also be used, with regeneration times ranging from 30 minutes to overnight.

Next step

Following column regeneration, immediately re-equilibrate the column with 3 to 5 CVs of equilibration buffer.

6. Column storage

Columns should be stored in PBS supplemented with 20% ethanol.

Note: Columns should be stored at +4°C.
Do not freeze!

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