Washing and Regenerating of Glutathione Agarose

Overview

Glutathione Agarose should be washed after each run, and preferably a given aliquot should only be used with the same protein. However, if the matrix becomes contaminated or shall be used with a different protein, a thorough cleaning procedure is recommended. This protocol delineates washing and regenerating procedures for PureCube Glutathione Agarose. Volumes are given in *column bed volume* (bv), i.e., 10 bv calls for 10 mL of buffer for a 1 mL column bed volume.
Please contact us if you have questions or need assistance optimizing a protocol for your application (contact@cube-biotech.com); other protocols can also be found at www.cube-biotech.com/protocols.

Equipment

- ☐ Disposable gravity flow columns with capped bottom outlet, 2 ml, (e.g. Pierce / ThermoScientific #29920)
- ☐ Alternatively, FPLC system and cartridges

Materials

- ☐ Tris-HCl, pH 7.4
- ☐ Sodium chloride (NaCl)
- ☐ Dithiothreitol (DTT)
- ☐ 20% (v/v) Ethanol

Suitable cleaning reagents:

- ☐ Sodium acetate
- ☐ Sodium hydroxide (NaOH)
- ☐ Hydrochloric acid (HCl)
- ☐ 70 % (v/v) Ethanol
- ☐ Sodium dodecyl sulfate (SDS)
- ☐ Triton X-100

Solutions and buffers

Wash Buffer, 100 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
Tris-HCl, pH 7.4	125 mM	121.14	0.5 M	30.29 g/500 mL	25 mL
NaCl	150 mM	58.44	5 M	146.1 g/500 mL	3 mL
DTT	1 mM	154.25	1 M	1.54 g/10 mL	100 µL
Instructions: Add water to a total volume of 100 mL.					

Procedure

Wash (recommended after each run)

1. After the elution step, add 10 bv Wash Buffer and allow the volume to completely flow through the matrix. The resin is now ready to be reused.

Tip: You can allow the fluid to drip through the column by gravity, or use a pressure bulb to gently force the fluid through the matrix.

Wash and store

1. After the elution step, add 10 bv Wash Buffer and allow the volume to completely flow through the matrix.
2. Rinse the column again with 10 bv water.
3. Finally, add 10 bv 20% (v/v) ethanol and allow the majority of the volume to drip out of the column. Store at 2-8°C.

Intensive cleaning

1. After the elution step, add 10 bv Wash Buffer and allow the volume to completely flow through the matrix.
2. Rinse the column again with 10 bv water.
3. Wash with **2 bv of one** of the following solutions. **Do not combine the chemicals!**

   1 M Sodium acetate, pH 4.0

   or 0.1 M Sodium hydroxide

   or 0.1 M Hydrochloric acid (HCl)

   or 70% (v/v) Ethanol

   or 1 % SDS

   or 1 % Triton X-100
4. Rinse the column again with 10 bv water.
5. Wash the column with 10 bv Wash Buffer
6. Repeat step 3-5 with a different chemical if necessary.
7. The resin is now ready to be reused.