Regenerating Rho1D4 Agarose

Overview

This protocol delineates a regenerating procedure for PureCube Rho1D4 Agarose to allow for re-use of the matrix. 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. Note that this protocol can be performed on a gravity flow column, but is much more convenient when done on an FPLC instrument.

Reusability of PureCube Rho1D4 Agarose depends on many factors: the protein purified, buffer conditions, storage conditions, etc.. Therefore it is hard to predict how many times the matrix can be regenerated without significant decrease in performance.

Please contact us if you have questions or need assistance optimizing a protocol for your application (contact@www.cube-biotech.com); other protocols can also be found at www.cube-biotech/protocols.

Equipment

- ☐ FPLC compatible column (e.g. PureCube Cartridge 1 mL, Cube Biotech #16911 or PureCube Cartridge 5 mL, Cube Biotech #16916, or larger columns)
- ☐ FPLC workstation (e.g. GE Äkta or BioRad BioLogic)
- ☐ Alternatively: Disposable gravity flow columns with capped bottom outlet, 2 ml, (e.g. Pierce / ThermoScientific #29920)

Materials

- ☐ Tris base
- ☐ Sodium acetate
- ☐ Hydrochloric acid (HCl)
- ☐ 20% (v/v) Ethanol

Solutions and buffers

High pH Regeneration Buffer, 500 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
Tris-base, pH 8.5	50 mM	121.14	1 M	60.57 g/ 500 mL	25 mL
NaCl	0.5 M	58.440	5 M	146.1 g/ 500 mL	50 mL
Instructions: Dissolve Tris base in 400 mL water, set the pH to 8.5 using HCl, and fill up to 500 mL. Use this stock solution to prepare the High pH Regeneration Buffer.					

Low pH Regeneration Buffer, 500 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
Sodium acetate, pH 4.5	100 mM	82.3	1 M	41.15 g/ 500 mL	50 mL
NaCl	0.5 M	58.440	5 M	146.1 g/ 500 mL	50 mL
Instructions: Dissolve sodium acetate in 400 mL water, set the pH to 4.5 using HCl, and fill up to 500 mL. Use this stock solution to prepare the Low pH Regeneration Buffer.					

Procedure

1. Wash the Rho1D4 Agarose for more than 10 times, alternating with 10 bv each of high pH and low pH Regeneration Buffer.
2. Check for efficient removal of Rho1D4 peptide by monitoring the absorption at 220 nm in the eluate. This value should be below 0.1 to ensure a complete regeneration.
3. Rinse the column with 10 bv water.
4. Finally, add 10 bv 20% (v/v) ethanol and allow the majority of the volume to drip out of the column. The resin is now ready to be reused.