Washing and Regenerating of HiCap StrepTactin Agarose

Overview

HiCap StrepTactin Agarose can be regenerated for re-use according to the protocol delineated below. Desthiobiotin is removed by an incubation with 2-(4-Hydroxyphenylazo)benzoic acid (HABA), followed by wash steps. 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. The material can be regenerated 3-5 times.
Note that a regeneration is only possible if desthiobiotin has been used for elution. If biotin has been used, binding to the agarose matrix is irreversible.
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 8.0
- ☐ Sodium chloride (NaCl)
- ☐ EDTA
- ☐ 2-(4-Hydroxyphenylazo)benzoic acid (HABA) (e.g. Sigma #H5126)
- ☐ Optional: Sodium azide
- ☐ Optional: Guandinium hydrochloride (GuHCl)

Solutions and buffers

Regeneration Buffer, 100 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
TRIS base, pH 8.0	100 mM	121.14	1 M	60.57 g/ 500 mL	10 mL
NaCl	150 mM	58.44	5 M	146.1 g/ 500 mL	3 mL
HABA	1 mM	242.23	-	24 mg	-
Instructions: Prepare a TRIS base stock solution and set the pH with HCl to 8.0. Alternatively, prepare 200 mL Wash Buffer and separate in 2 x 100 mL, add HABA to one aliquot.					

Wash Buffer, 100 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
TRIS base, pH 8.0	100 mM	121.14	1 M	60.57 g/ 500 mL	10 mL
NaCl	150 mM	58.44	5 M	146.1 g/ 500 mL	3 mL
Instructions: Prepare a TRIS base stock solution and set the pH with HCl to 8.0.					

Storage Buffer, 100 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
TRIS base, pH 8.0	100 mM	121.14	1 M	60.57 g/ 500 mL	10 mL
EDTA	1.3 mM	292.24	0.5 M	14.6 g/100 mL	260 µL
Sodium azide	0.02%	-	-	-	20 mg
Instructions: Prepare a TRIS base stock solution and set the pH with HCl to 8.0.					

Procedure

1. After the elution step, add 5 bv Wash Buffer and allow the volume to completely flow through the matrix.
2. Optional: To remove protein aggregates, wash the column with 2 bv of 6 M guanidinium hydrochloride, followed by two wash steps with 4 bv each of wash buffer.
3. Wash the column three times with 5 bv Regeneration buffer each.
4. Wash the column twice with 4 bv Wash Buffer each, or until it has completely turned white again.
5. Store the HiCap StrepTactin Agarose in Wash Buffer at 4°C. for several days.
6. For long-term storage, wash twice with 4 bv Storage Buffer and store the HiCap StrepTactin Agarose.

Important: HABA in solution has a yellow color that changes to red upon interaction with the StrepTactin matrix. This indicates a displacement of desthiobiotin. The red color disappears upon washing with wash buffer. Wash until the column is completely white again.