

Octave™ Ni-NTA SF Columns



Cat. Nos. C1001S & C1002S

For more detailed information please refer to the Semba Biosciences website www.sembabio.com.

DESCRIPTION

Octave Ni-NTA SF Columns are pre-filled with 1 ml or 5 ml Ni-NTA Superflow and are ready for purification of His-fusion proteins with Semba's Octave™ Chromatography System. The His-fusion tag comprising 6 or more consecutive histidine residues binds with high selectivity to the nickel-nitrilotriacetic acid (Ni-NTA) resin and provides significant advantages for affinity purification of recombinant proteins. Recommended conditions for the Octave System have been scripted in the SembaPro™ software for convenient purification with either 1-ml or 5-ml Octave Ni-NTA SF columns. The Ni-NTA SF matrix has excellent mechanical stability, flow characteristics and high dynamic binding capacity, with up to 50 mg His-fusion protein bound per milliliter.

The Superflow resin is a highly cross-linked 6% agarose with a bead diameter of 60-169 µm. The column body is composed of polypropylene. Connectors are 1/16", 10-32 (inlet) and M6 (outlet).

USE WITH THE OCTAVE CHROMATOGRAPHY SYSTEM

Octave Ni-NTA SF Columns are ideally suited for use on the Octave Chromatography System. The System User Manual describes these procedures in detail, including preparation of protein samples from bacterial cultures. For optimal results it is important to estimate the total protein and target protein concentration in the cell lysate that will be used for purification. The Semba Biosciences Express-timator™ Kit can be used to estimate target protein expression levels.

OPERATIONAL GUIDELINES

The guidelines below provide a starting point for obtaining purified His-fusion proteins using Octave Ni-NTA SF columns.

1. Standard affinity purification buffers (also used for Step-mode operation on the Octave System): Bind, 50 mM sodium phosphate, 300 mM sodium chloride, 10 mM imidazole, pH 8.0; Wash, 50 mM sodium phosphate, 300 mM sodium chloride, 20 mM imidazole, pH 8.0; and Elute, 50 mM sodium phosphate, 300 mM sodium chloride, 250 mM imidazole, pH 8.0.
2. Octave System Isocratic mode buffer: 50 mM sodium phosphate, 300 mM sodium chloride, 75 mM imidazole, pH 8.0.
3. All buffers and cell lysates must be filtered using a 0.8 µm or smaller pore size filter.
4. Recommended feed flow rates are 1 ml/min for 1-ml columns, and 5 ml/min for 5-ml columns. Maximum flow rates are 10 ml/min and 40 ml/min for 1-ml and 5-

ml columns, respectively. Maximum pressure is 5 bar (0.5 MPa, 72.5 psi) for both sizes,

5. Equilibration and wash steps are accomplished with at least 5 column volumes of buffer. Elution of the purified protein is within 3-5 column volumes.

We recommend using SembaChrom™ Grade Imidazole for preparation of solutions; this highly purified reagent has been processed to reduce UV-absorbing contaminants that interfere with protein readings at 280 nm.

COMPATIBILITIES AND LIMITATIONS

Octave Ni-NTA SF Columns are compatible with buffers containing up to 10 mM DTT. Up to 20 mM β-mercaptoethanol can also be used as an alternative reducing agent; however, the resin will turn reversibly brown due to nickel reduction.

Other reagents shown to be compatible with His-fusion protein/Ni-NTA interaction include 2% Triton X-100, 2% Tween 20, 1% CHAPS, 1X SembaSonic™ Protein Extraction Reagent, 50% glycerol, 4 M MgCl₂, 5 mM CaCl₂, and 2 M NaCl.

Strong metal chelating agents, such as EDTA and EGTA, bind nickel ions and strip them from the NTA matrix. The resin will turn white and will not bind His-fusion proteins. The resin must be recharged with 100 mM NiSO₄ to regain functionality if stripping has occurred.

CLEANING

If an increase in back pressure or significant contamination of the resin is observed, a cleaning-in-place (CIP) procedure, which usually fully restores performance, can be performed. Due to the low metal-leaching rate of Ni-NTA, stripping is not required prior to the CIP procedure. It is recommended to use the following protocol to remove contaminants such as precipitated proteins, hydrophobically bound proteins, and lipoproteins.

Wash the columns with 15 volumes of 0.5 M NaOH. Wash 1-ml columns at a flow rate of 0.5 ml/min for 30 min. Wash the 5-ml columns at a flow rate of 2.5 ml/min for 30 min.

Re-equilibrate the columns with 10 volumes of buffer. The columns are ready for use.

STORAGE

Octave Ni-NTA SF Columns should be stored at 2-8°C. Do not freeze! Columns can be stored up to 1 week in Bind buffer. Columns can be stored in 20% ethanol (as supplied) for one year without any reduction in performance.

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