The Semba Octave™ Chromatography System

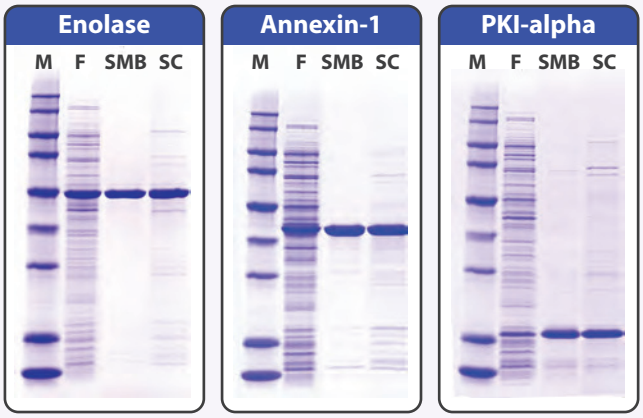
The Power of Simulated Moving Bed Chromatography on Your Bench

Application Note 2: Continuous Affinity Purification of Recombinant Proteins

Recombinant protein purification using affinity media in a traditional single-column (SC) elution chromatography mode is a well established technique. Usually, recombinant proteins are expressed with N- or C-terminal fusion tag such as a hexahistidine peptide and purified by immobilized metal affinity chromatography (IMAC). For many proteins, the level of purity achieved with the SC-IMAC step alone is not sufficient to allow further studies, such as functional characterization, X-ray crystallography, or drug target validation. In these cases additional purification steps are required which result in higher cost, delays and lower yields. Improvements such as increased purity and efficiency at any of these steps, IMAC in particular, would increase the throughput and lower the cost of purification.

Continuous countercurrent simulated moving bed chromatography (SMBC) has been gaining acceptance as an efficient method for chiral separation (1). However, there are a very few publications reporting adoption of SMBC for bioseparations (2, 3), perhaps due to the lack of suitable instrumentation. Here we demonstrate the utility of bench top SMBC for continuous affinity purification of several human 6xHis-fusion proteins expressed in *E. coli*. Human Enolase, Annexin-1, and PKI-alpha were purified using Ni-chelate columns and the Semba Octave System in a novel "isocratic affinity" mode. A comparison with single column purification revealed that the SMBC process resulted in significant gains in purity. In addition, the continuous SMBC process produced several hundred milligrams of purified protein from eight 1-ml cartridges in a few hours of unattended operation.

Columns: 8 x 1 ml Ni-agarose or Ni-methacrylate cartridges
Samples: bacterial cell lysates containing 6xHis-fusion proteins
Purification: 3-2-3 SMBC vs. standard batch single column
M = size markers
F = Feed (clarified *E. coli* lysate)
SMB = SMBC-purified
SC = Single column-purified



Purity

	SMBC	Single Col.
Enolase	96%	89%
Annexin	83%	66%
PKI-alpha	69%	44%

SMBC Yield (8 x 1 ml columns)

	Feed Input	Total Product	mg/h
Enolase	213 ml	246 mg	55
Annexin	162 ml	231 mg	68

Purification of recombinant proteins from bacterial lysates by immobilized metal affinity chromatography (SMB-IMAC)

The indicated human proteins were expressed in *E. coli* as fusion proteins tagged with a hexahistidine peptide to enable their purification by immobilized metal affinity chromatography. Crude *E. coli* lysates were prepared by standard methods and applied to Ni-chelate columns on the Semba Octave System in a 3-2-3 isocratic configuration and to an identical single column. The single columns were processed manually per manufacturers' instructions. Samples of Feed and purified products were analyzed by SDS-polyacrylamide gel electrophoresis and Coomassie blue staining. Equivalent amounts of protein were loaded for each pair of purified samples.

1. Juza, M. (2000) *TIBTECH* **18**, 108-118.
2. Gottschlich, N. (1997) *J. Chromatogr. A* **765**, 201-206.
3. Voigt, U. (1996) German patent DE 19611094A1

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