

Cation Exchange Procedure for ICAT Samples

HPLC columns: PolyLC Inc.

1) 2.1 mm x 20 cm, 5 μ m particles, 300 \AA pore size, Polysulfoethyl A strong cation exchange material. Manufacturer's suggested protein max: 1mg protein for good chromatography; 5mg is max.

2) 4.6 mm x 20 cm, 5 μ m particles, 300 \AA pore size Polysulfoethyl A strong cation exchange material. Manufacturer's suggested protein max: 5mg protein for good chromatography; 30mg max.

Buffers:

Buffer A

5 mM K_2HPO_4 , 25% CH_3CN , pH 3.0

Buffer B

5 mM K_2HPO_4 , 25% CH_3CN , 350 mM KCl , pH 3.0

Gradient:

<u>Time (min)</u>	<u>%B</u>
0	0
30	25
50	100

Other Notes:

The flow rate is 200 μ l per minute for the 2.1 mm column and 800 μ l per minute for the 4.6 mm column. Fractions are collected at 1 minute intervals. We have loaded anywhere from about 120 μ g up to about 5 mg of digested, ICAT labeled total protein on the 2.1 mm column, and up to 30 mg on the 4.6 mm column. The samples are usually diluted 1:1 with Buffer A, pH 2.5-3.0. **It is important to acidify the samples down to pH 3.0 or below before loading onto the cation exchange because peptides will not be fully charged at higher pH values and may not stick to the column!** A sample loop with a

2 ml capacity is loaded with sequential 2 ml portions of the total volume of the sample. The column and loop are then washed with Buffer A until the baseline returns to approximately zero before loading the next 2 mls (about 15 minutes between each loading). The gradient shown is designed to spread out the elution of doubly-charged peptides as much as possible, with these peptides usually eluting starting at about 8-9 minutes into the run until approximately minutes 15-16, after which triply charged peptides begin to elute.