



Analysis of mAb using a pH Gradient on Proteomix® SCX NP5 4.6 x 250 mm

APPLICATION NOTES

Ion Exchange Chromatography is frequently used for antibody analysis. Antibodies and antibody fragments can all be separated on cation exchange columns based on their charge states.

Sepax's Proteomix® SCX is a complementary option to the Antibodix™ WCX phase for the high resolution, high efficiency and high recovery analysis of antibodies and their variants.

Highlighted FACTS:

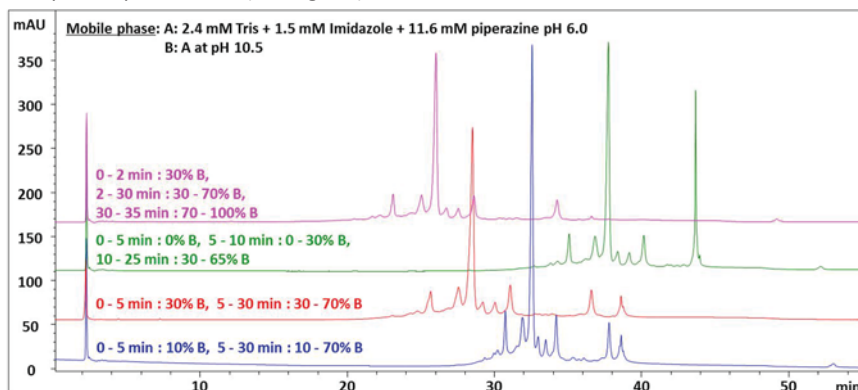
- »» Proteomix® SCX NP5 4.6 x 250 mm can successfully separate monoclonal antibody variants using a pH gradient.
- »» Monoclonal antibody purity, heterogeneity and stability can be monitored using Proteomix® SCX NP5.
- »» The 5 µm particle size in Proteomix® SCX NP5 offers superior resolution.
- »» High stability packing material allows for analyses in wide pH and temperature ranges.

Optimization of pH Gradient for mAb Analysis on Proteomix® SCX

Column: Proteomix® SCX NP5 (5 µm, 4.6 x 250 mm)

Flow rate: 0.8 mL/min, Detector: UV 280 nm, Column temperature: 25 °C

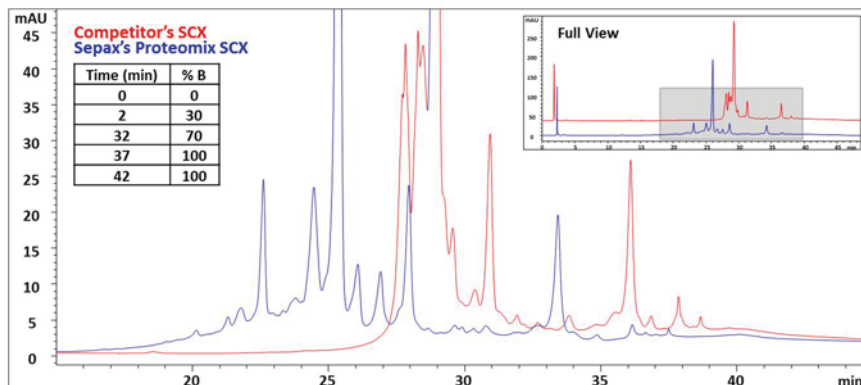
Sample: 10 µL mAb 321 (5.0 mg/mL)



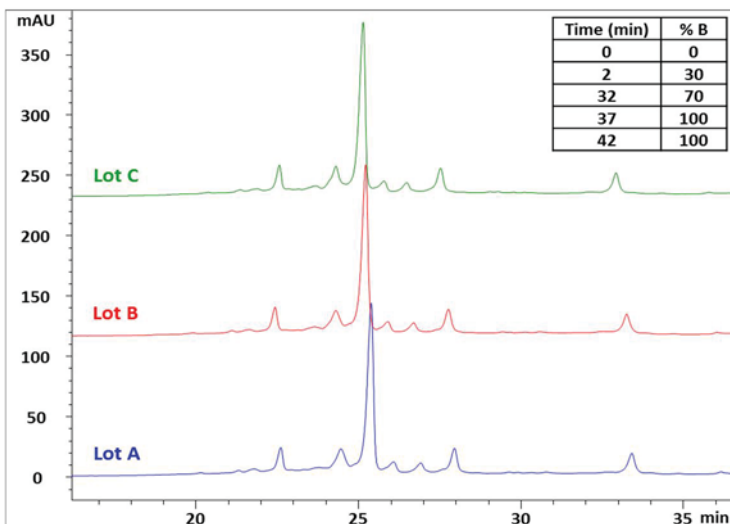
Analysis of mAb on Proteomix® SCX Compared to Competitor's SCX

Columns: Proteomix® SCX NP5 (4.6 x 250 mm) and Competitor's SCX (5 µm, 4.0 x 250 mm)

Mobile phase: A: 2.4 mM Tris + 1.5 mM Imidazole + 11.6 mM piperazine pH 6.0 and B: A at pH 10.5; Flow rate: 0.8 mL/min, Detector: UV 280 nm, Column temp: 25 °C, Sample: 10 µL mAb 321 (5 mg/mL)

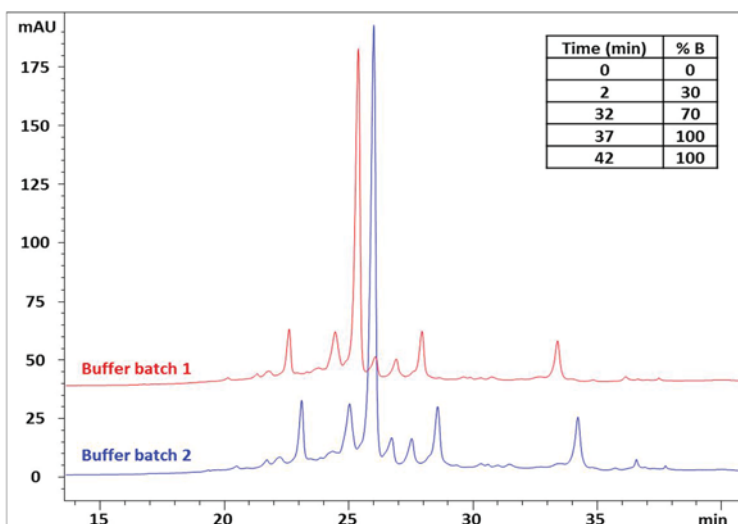


Analysis of mAb 321 on Proteomix® SCX NP5 Lot to Lot Comparison to show Reproducibility



Column: Proteomix® SCX NP5 (4.6 x 250 mm), Mobile phase: A: 2.4 mM Tris + 1.5 mM Imidazole + 11.6 mM piperazine pH 6.0 and B: A at pH 10.5; Flow rate: 0.8 mL/min, Detector: UV 280 nm, Column temperature: 25 °C, Sample: 10 µL mAb 321 (5 mg/mL)

Analysis of mAb 321 on Proteomix® SCX NP5 Reproducibility between batches of Mobile Phase





Analysis of mAb using a pH Gradient on Proteomix® SCX NP5 4.6 x 250 mm

What is Proteomix® SCX NP5

Proteomix® SCX NP5 (Strong Cation Exchange):

Comprised of rigid, spherical, highly cross-linked non-porous poly(styrene divinylbenzene) (PS/DVB) beads. The PS/DVB particle surface is grafted with a hydrophilic, neutral polymer layer which is nanometers thick. The resin surface is covered by a hydrophilic coating which eliminates non-specific bindings with antibody proteins, leading to high efficiency and high recovery separations. On top of the hydrophilic layer, strong cation-exchange sulfonate ($-\text{SO}_3\text{H}$) functional groups are attached via a proprietary chemistry, resulting in a high capacity ion-exchange layer.

Technical Specifications:

Phase	Proteomix® SCX NP5
Material	Sulfonate strong cation exchange groups bonded to a hydrophilic film grafted on PS/DVB
Particle size (μm)	5
Pore size (Å)	Non-porous
pH stability	2 – 12
Backpressure (psi)	~ 3,500
Maximum backpressure	~ 6,000
Maximum temperature	~ 80 °C
Mobile phase compatibility	Aqueous or a mixture of water and acetonitrile, acetone or methanol

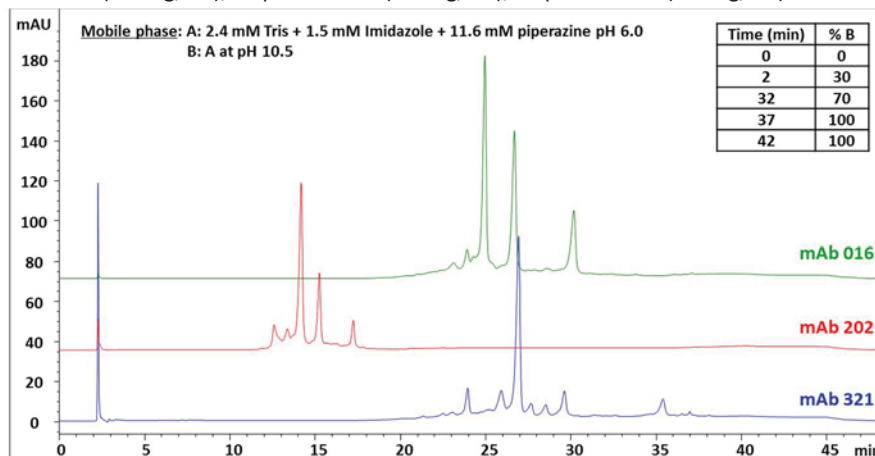
For ordering information, please visit:
www.sigmaaldrich.com/sepax

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Three Different mAbs on Proteomix® SCX using the same pH Gradient

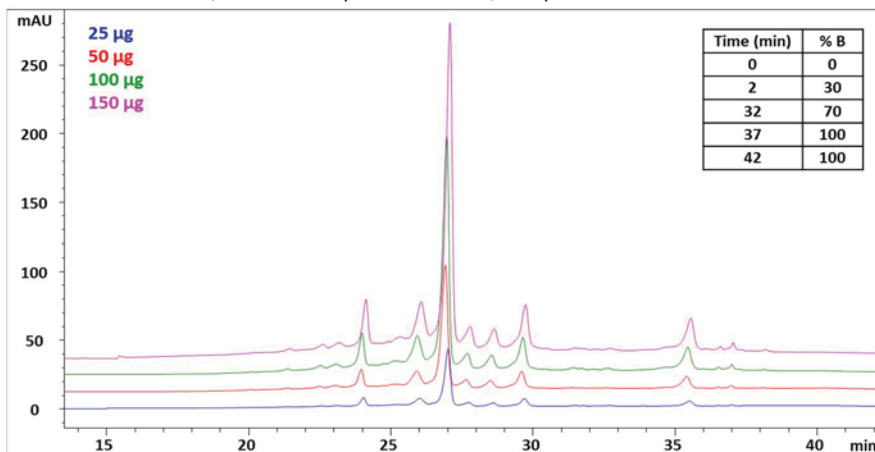
Column: Proteomix® SCX NP5 (5 μm , 4.6 x 250 mm)

Flow rate: 0.8 mL/min, Detector: UV 280 nm, Column temperature: 25 °C, Sample: 10 μL mAb 321 (5.0 mg/mL), 50 μL mAb 202 (1.0 mg/mL), 10 μL mAb 016 (5.9 mg/mL)



Loading Test for mAb 321 on Proteomix® SCX using a pH gradient

Column: Proteomix® SCX NP5 (5 μm , 4.6 x 250 mm), Mobile phase: A: 2.4 mM Tris + 1.5 mM Imidazole + 11.6 mM piperazine pH 6.0 and B: A at pH 10.5; Flow rate: 0.8 mL/min, Detector: UV 280 nm, Column temperature: 25 °C, Sample: mAb 321



Faster mAb Analysis on Proteomix® SCX with a Higher Flow Rate

Column: Proteomix® SCX NP5 (5 μm , 4.6 x 250 mm), Mobile phase: A: 2.4 mM Tris + 1.5 mM Imidazole + 11.6 mM piperazine pH 6.0 and B: A at pH 10.5; Flow rate: specified, Detector: UV 280 nm, Column temperature: 25 °C, Sample: 10 μL mAb 321 (5.0 mg/mL)

