Hydrophobic Interaction Chromatography Column

BioPro HIC BF

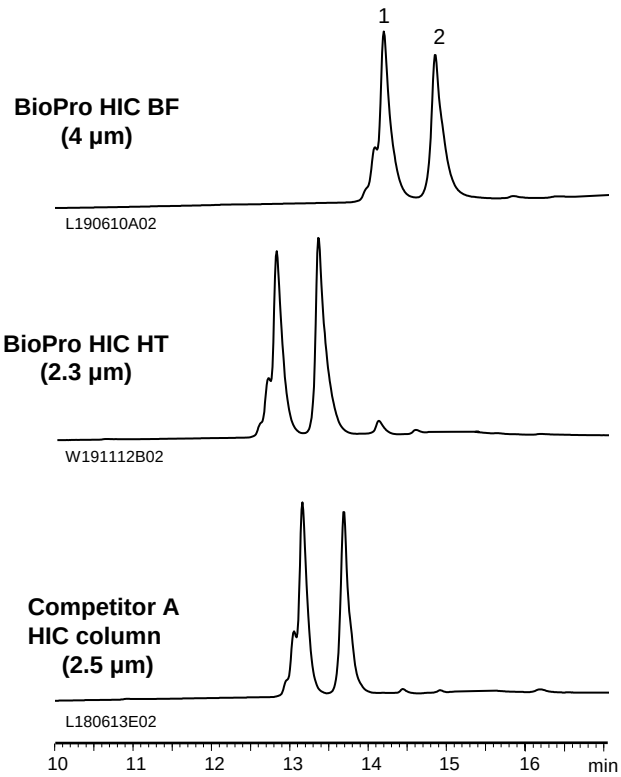
Features

- **Capable of purifying proteins such as antibodies without the denaturation**
- **Designed for separation of low hydrophobic proteins**

Specification

Matrix	: Hydrophilic non-porous polymer
Particle size	: 4 µm
Bonded phase	: Butyl group
Usable temp. range	: 10-60℃
Usable pH range	: 2-12
Pressure limit	: 20 MPa

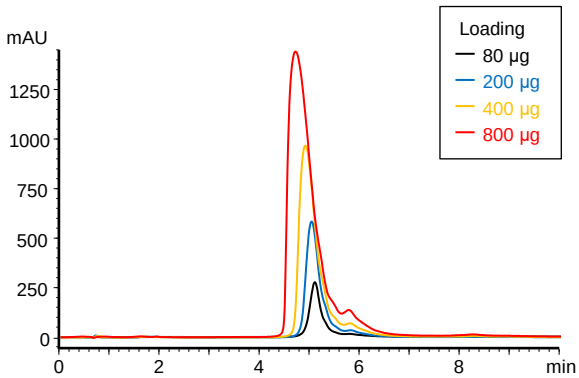
Higher hydrophobicity of HIC stationary phase



Column	: 100 X 4.6 mmI.D.
Eluent	: A) 100 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0) containing 2.0 M $(NH_4)_2SO_4$ B) 100 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0) 0%B (0-1 min), 0-100%B (1-11 min), 100%B (11-15 min)
Flow rate	: 0.5 mL/min
Temperature	: 25℃
Detection	: UV at 280 nm
Injection	: 15 µL
Sample	: 1. Adalimumab (0.5 mg/mL) 2. Trastuzumab (0.5 mg/mL)

BioPro HIC BF shows the stronger retention of proteins due to the higher hydrophobicity of its stationary phase compared to the other commercially available HIC columns. This indicates that BioPro HIC BF could be preferred for the separation of low hydrophobic proteins.

Excellent peak shape under high loading condition



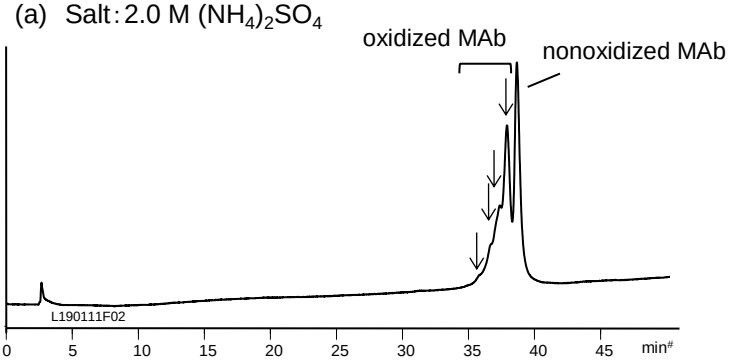
Column	: BioPro HIC BF 4 µm, 100 X 4.6 mmI.D.
Eluent	: A) 100 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0) containing 2.0 M $(NH_4)_2SO_4$ B) 100 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0) 60%B (0-0.5 min), 60-100%B (0.5-7.5 min), 100%B (7.5-10 min)
Flow rate	: 1.2 mL/min
Temperature	: 30℃
Detection	: UV at 280 nm
Sample	: Humanized monoclonal IgG (2.5 mg/mL)

BioPro HIC BF shows excellent peak shape even under high loading conditions. This leads to effective for laboratory-scale purification and detection of minor constituents by the large volume injection.

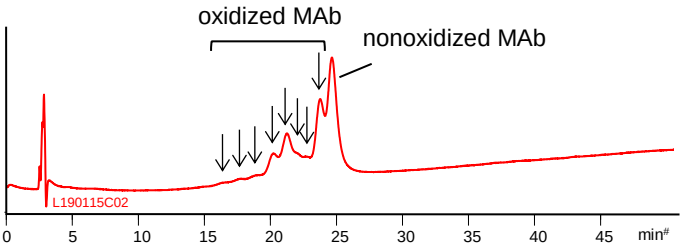
Capable of evaluating MAb oxidization in HIC

Analysis of oxidized MAb

(a) Salt: 2.0 M $(NH_4)_2SO_4$



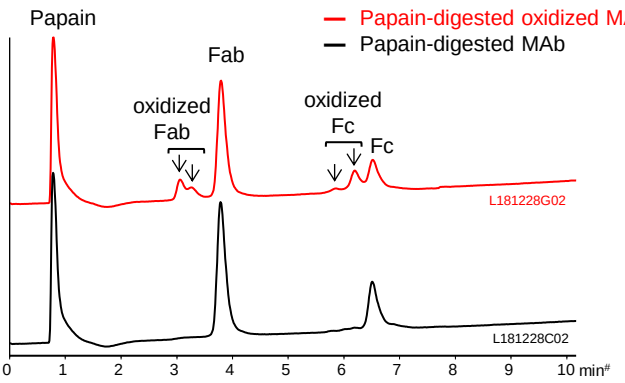
(b) Salt: 4.0 M NaCl



Column	: BioPro HIC BF 4 µm, 100 X 4.6 mmI.D.
Eluent	: A) 100 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0) containing salt B) 100 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0) 40-80%B (0-40 min), 80%B (40-45 min)
Flow rate	: 0.3 mL/min
Temperature	: 25℃
Detection	: UV at 280 nm
Injection	: 5 µL (1.0 mg/mL)

NISTmAb was treated with *tert*-butyl hydroperoxide (*t*-BHP) as an oxidant in order to promote the oxidation. The oxidized MAb was analyzed by using BioPro HIC BF column.
Under the ammonium sulfate condition (a), four peaks appeared at earlier elution times compared to the peak of the nonoxidized MAb, presumably due to the conformational changes via the oxidization of the methionine residues.
Under the sodium chloride condition (b), eight peaks appeared at earlier elution times compared to the peak of the nonoxidized MAb. Such a better resolution was achieved with the shorter analysis time compared with under the ammonium sulfate condition (a).

Analysis of papain-digested oxidized MAb



Column	: BioPro HIC BF 4 µm, 100 X 4.6 mmI.D.
Eluent	: A) 100 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0) containing 2.0 M $(NH_4)_2SO_4$ B) 100 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0) 40-80%B (0-10 min)
Flow rate	: 1.0 mL/min
Temperature	: 25℃
Detection	: UV at 280 nm
Injection	: 5 µL (0.5 mg/mL)

The papain-digests of NISTmAb samples with and without the oxidization were analyzed by using BioPro HIC BF column. The Fab and Fc fragments were characterized from the chromatogram of the papain-digested MAb.
In the chromatogram of the papain-digested oxidized MAb, the multiple peaks appeared at earlier elution times compared to the peaks assigned to the Fab and Fc fragments, and would be corresponding to the oxidized fragments. According to the previous report*, the oxidized fragments elute earlier than the nonoxidized ones.

*Journal of Chromatography A, 2008, 1214, 81-89

【Ordering information】

Particle size (µm)	Column size Inner diameter X length (mm)	Product number
4	4.6 X 100	BHB00S04-1046WT

Please contact us about the product with other size. Preparative columns are also available.

Worldwide Availability

YMC America, Inc. www.ymcamerica.com
YMC Europe GmbH www.ymc.de
YMC Switzerland LLC www.ymc-schweiz.ch
YMC Shanghai Rep. Office www.ymcchina.com
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