

# 04

## Hydrophobic Interaction Chromatography Column

BioPro HIC BF----- 38-39

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## BioPro HIC BF

- Effective for separation of proteins and antibodies such as ADCs
- High resolution comparable to sub-3  $\mu\text{m}$  with low operating pressure
- Usable for laboratory scale purification

- Particle size : 4  $\mu\text{m}$
- Usable pH range : 2-12

### Hydrophobic interaction chromatography column for separation of biologics

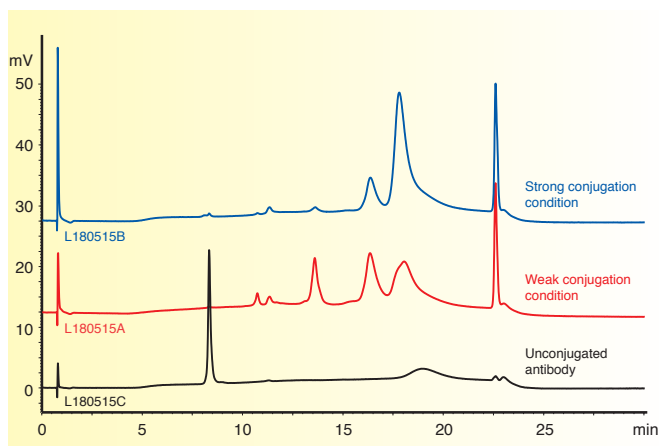
BioPro HIC BF is based on a non-porous hydrophilic polymer. It is designed and optimized for analysis and laboratory scale purification of antibodies, antibody-drug conjugates (ADCs) and other proteins.

Optimized surface modification and column packing method realize high separation performance. High-throughput analysis with a faster flow rate can be achieved by the rigid particle and moderate back pressure.

### Specifications

|                                 | BioPro HIC BF                  |
|---------------------------------|--------------------------------|
| Matrix                          | Hydrophilic non-porous polymer |
| Particle size ( $\mu\text{m}$ ) | 4                              |
| Bonded phase                    | Butyl group                    |
| Usable temperature              | 10-60°C                        |
| Usable pH range                 | 2-12                           |

### Effective for Drug-to-Antibody Ratio (DAR) analysis of ADCs

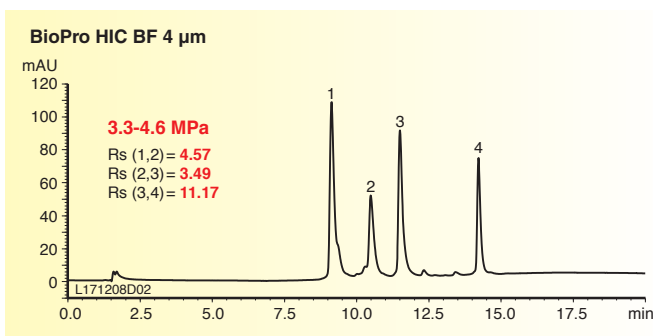


Column : BioPro HIC BF 4  $\mu\text{m}$ , 100 X 4.6 mm I.D.  
 Eluent : A) 50 mM  $\text{NaH}_2\text{PO}_4$ - $\text{Na}_2\text{HPO}_4$  (pH 7.0) containing 1.5 M  $(\text{NH}_4)_2\text{SO}_4$ /2-propanol (95/5)  
 B) 50 mM  $\text{NaH}_2\text{PO}_4$ - $\text{Na}_2\text{HPO}_4$  (pH 7.0)/2-propanol (80/20)  
 0%B (0-1 min), 0-100%B (1-15 min), 100%B (15-20 min), 0%B (20-30 min)  
 Flow rate : 1.0 mL/min  
 Temperature : 25°C  
 Detection : UV at 280 nm  
 Sample : Antibody Drug Conjugate\*

\*Courtesy by RIKEN.

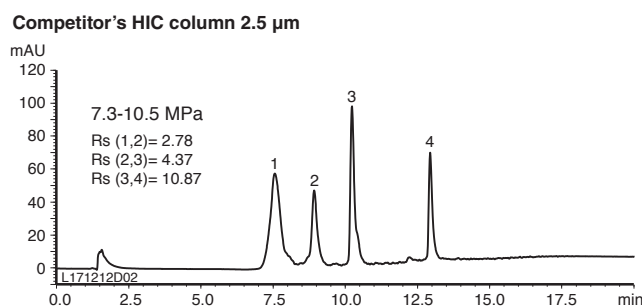
ADCs with different conjugation condition were analyzed using BioPro HIC BF. These results indicate the increase of ADC with high DAR under the strong condition compared to the weak condition. BioPro HIC BF column provides superior separation for ADCs, therefore it is effective for DAR determination and monitoring of conjugation reactions.

## High resolution comparable to sub-3 $\mu\text{m}$ with low operating pressure



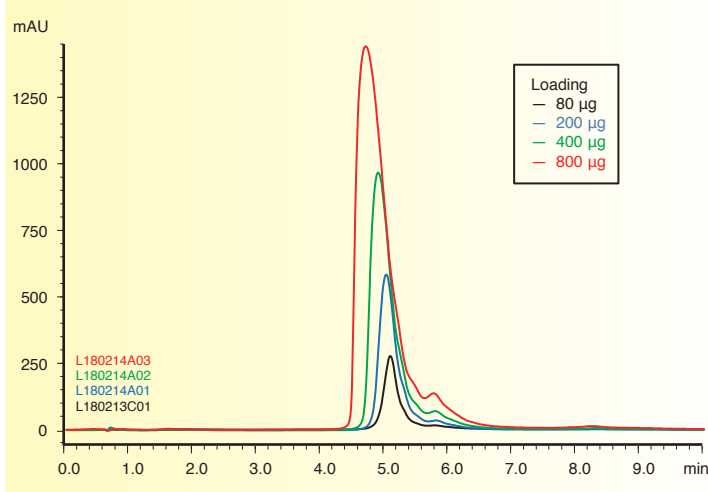
1. Myoglobin
2. Ribonuclease A
3. Lysozyme
4.  $\alpha$ -Chymotrypsinogen A

Column : 100 X 4.6 mm I.D.  
 Eluent : A) 100 mM  $\text{NaH}_2\text{PO}_4$ - $\text{Na}_2\text{HPO}_4$  (pH 7.0) containing 2.0 M  $(\text{NH}_4)_2\text{SO}_4$   
 B) 100 mM  $\text{NaH}_2\text{PO}_4$ - $\text{Na}_2\text{HPO}_4$  (pH 7.0) 0-100%B (0-11 min), 100%B (11-15 min)  
 Flow rate : 0.5 mL/min  
 Temperature : 25°C  
 Detection : UV at 280 nm  
 Injection : 15  $\mu\text{L}$



The separation of proteins is compared on BioPro HIC BF with particle size 4  $\mu\text{m}$  and a competitor's HIC column with particle size 2.5  $\mu\text{m}$ . BioPro HIC BF with optimized media design and packing technology has superior resolution comparable to competitor's column with sub-3  $\mu\text{m}$ .

## Excellent peak shape under high loading



Column : BioPro HIC BF 4  $\mu\text{m}$ , 100 X 4.6 mm I.D.  
 Eluent : A) 100 mM  $\text{NaH}_2\text{PO}_4$ - $\text{Na}_2\text{HPO}_4$  (pH 7.0) containing 2.0 M  $(\text{NH}_4)_2\text{SO}_4$   
 B) 100 mM  $\text{NaH}_2\text{PO}_4$ - $\text{Na}_2\text{HPO}_4$  (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°C  
 Detection : UV at 280 nm  
 Sample : Humanized monoclonal IgG (2.5 mg/mL)

BioPro HIC BF provides superior peak shapes, even under high loading conditions. This allows detection of very tiny amounts of impurities in the sample. In addition, it can be used for laboratory scale purifications, e.g. for isolation of variants for various research requirements (e.g. structural analysis).

## Ordering Information –Column–

### BioPro HIC BF

| Particle size ( $\mu\text{m}$ ) | Column size inner diameter X length (mm) | Product number  |
|---------------------------------|------------------------------------------|-----------------|
| 4                               | 4.6 X 100                                | BHB00S04-1046WT |