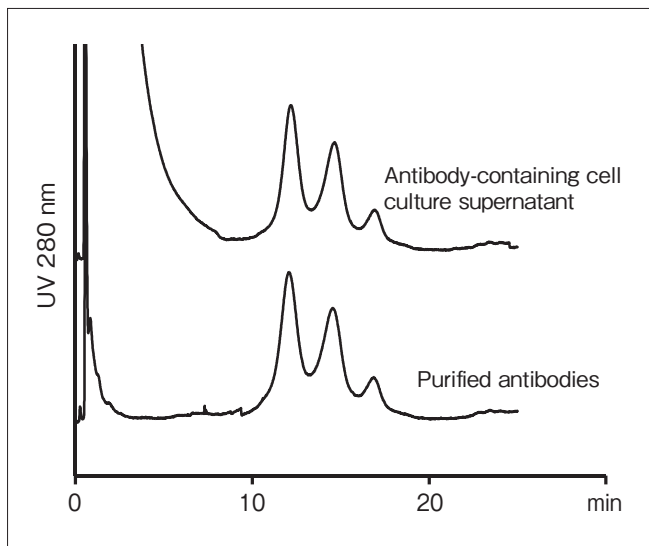


SEPARATION REPORT

TSKgel[®] FcR-III A-NPR Column for High-Performance Affinity Chromatography

— Table of Contents —

	Page
1. Introduction	1
2. Basic characteristics	1
2-1 Packing material properties	1
2-2 Standard separation conditions	1
2-3 Effect of eluent composition	3
2-4 Effect of sample load	4
2-5 Durability	4
2-6 Cleaning method	5
2-7 Lot-to-lot variance of packing materials	5
2-8 Stability in storage	6
3. Applications	6
3-1 Antibody applications	6
3-2 Relationship of antibody glycan structure and retention ability	7
3-3 Analysis of antibodies within cell culture supernatant	7
4. Precautions for use	8
5. Conclusion	8



<Conditions>

Column: TSKgel FcR-III A-NPR (4.6 mm I.D. × 7.5 cm)

Eluent A: 50 mmol/L sodium citrate buffer +

150 mmol/L NaCl (pH 6.5)

B: 50 mmol/L sodium citrate buffer +

150 mmol/L NaCl (pH 4.5)

Gradient: 0 - 7 min B 0 %

7 - 25 min B 0 % to 100 %, linear gradient

25 - 30 min B 100 %

30 - 35 min B 0 %

Flow rate: 1.0 mL/min

Detection: UV 280 nm

Temperature: 20°C

Sample: Antibody-containing cell culture supernatant, purified antibody

Figure 10. Comparison of separations of antibody-containing cell culture supernatant and purified antibody

4. Precautions for use

Table 4 lists precautions for use.

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HPLC system	<p>1. Internal system cleaning before use (perform prior to connecting the column)</p> <p>1) Systems where the internal system cleaning is sufficient Send 0.1 mol/L citric acid solution through at 1.0 mL/min for approximately 30 minutes.</p> <p>2) Old systems and systems where internal system cleaning is insufficient (1) Send 0.1 mol/L sodium hydroxide solution through at 1.0 mL/min for approximately 30 minutes. (2) Send purified water through at 1.0 mL/min for approximately 30 minutes. (3) Send 0.1 mol/L citric acid solution through at 1.0 mL/min for approximately 30 minutes.</p> <p>After performing either of the aforementioned cleanings in 1) and 2), replace liquid phase with eluent A and send the liquid through at 1.0 mL/min for approximately 15 minutes, and then run a blank gradient. Subsequently, connect and equilibrate the column. After running a blank gradient, start performing analyses.</p> <p>2. Line filter Installing a line filter between the pump and injector is recommended. · Line filter kit PEEK (Product No.: 0018014)</p> <p>3. Column oven equipped with cooling function The column operating temperature is 15 to 25°C. Because the elution time for samples is affected by temperature, it is recommended to perform analyses while maintaining a constant temperature with a column oven equipped with a cooling function.</p>
Eluent	When using a citrate buffer solution as the eluent, preparing the buffer solution from citric acid and sodium citrate is recommended. After preparing the eluent, filtering of the eluent with a 0.2- μ m-pore-size filter is recommended. In addition, because microorganisms easily grow in the eluent, it is recommended to prepare eluent at the time of use.
Sample solution	It is recommended to inject sample solution filtered with a 0.2- μ m-pore-size filter.
Column cleaning	Inject eluent containing 0.5 mol/L NaCl or eluent containing 20% ethanol multiple times from the injector.
Column storage	After replacing with preloaded solvent (0.025% ProClin [®] 300 + 0.65 mmol/L citric acid + 9.35 mmol/L trisodium citrate (pH 6.5)), place in refrigerated storage (2 to 8°C).
Column expiration date	The column has an expiration date. The expiration date is noted on the column box and in the Analysis Report accompanying the column.

5. Conclusion

We have presented an overview of the new TSKgel FcR-III A-NPR column intended for antibody drugs. Conventionally, expensive equipment and complicated work operations were required to analyze the glycan structure or to evaluate the activity of antibody drugs. However, the new analysis methods

using this column are extremely easy and can provide results with good reproducibility in a short amount of time. In addition, this column can be used not only for antibody drug quality control, but also for screening of cell strains for production use, optimization of the composition of cell culturing medium, process analysis of culturing processes, and the like.

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