PERFORMANCE DATA

ROBUSTNESS AND DYNAMIC RANGE OF
TSKgel FCR-IIIA-NPR AFFINITY COLUMN

TSKgel FcR-IIIA-NPR is a 5 µm, 4.6 mm ID × 7.5 cm analytical column for high performance affinity chromatography. It is based on a recombinant FcγIIIa receptor ligand immobilized on a non-porous polymer particle. It allows fast assessment of biologic activity of monoclonal antibodies.

FcγIIIa receptor plays a key role in antibody-dependent cell-mediated cytotoxicity (ADCC) by interacting with the N-glycans of IgG Fc regions. Hence, affinity chromatography on Fc receptor ligands can deliver valuable information about expected ADCC activity and mAb glycoform distribution. This fast and efficient method can be applied to purified samples and supernatant alike.

This note shows that TSKgel FcR-IIIA-NPR is very robust and allows analysis of a large number of injections with the same method. In addition to robustness we analyzed the dynamic range of the column. The results show that this HPLC method is suited for concentration ranges of many potential applications such as cell line screening in early R&D, biosimilar/originator comparison, upstream development and optimization, monitoring of glycoengineering, or lot-to-lot comparison in QC.

ROBUSTNESS

The TSKgel FcR-IIIA-NPR column is stable over 200 injections of a therapeutic mAb from CHO cell culture, as shown in Figure 1. The results demonstrate that impurities, such as host cell protein, do not impact the analysis. The 200 injection stability utilized addition of sodium chloride to minimize unwanted non-specific interactions and yielded good durability at 20 °C.

DYNAMIC RANGE

The sample loading linearity of the TSKgel FcR-IIIA-NPR column is shown in Figure 2. Because the column can load from 5-50 µg of IgG, it provides a suitable range for quantitative analysis of the antibody. Excellent correlation for the three major peaks in the elution profile was obtained for this concentration range. Column loading was varied at a constant injection volume for a trastuzumab biosimilar. The limits of detection and quantitation are 2.0 and 5.0 µg on column, respectively.
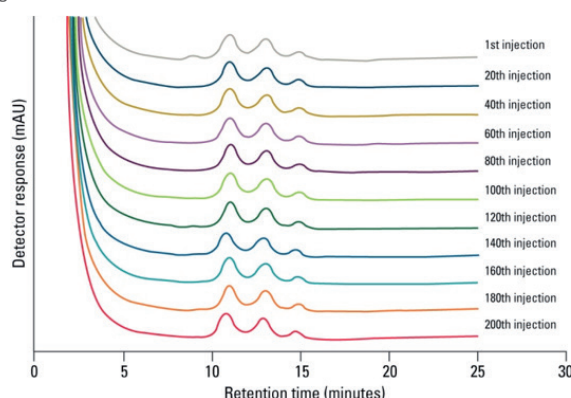
TSKgel FCR-IIIA-NPR COLUMN STABILITY

➤ Figure 2

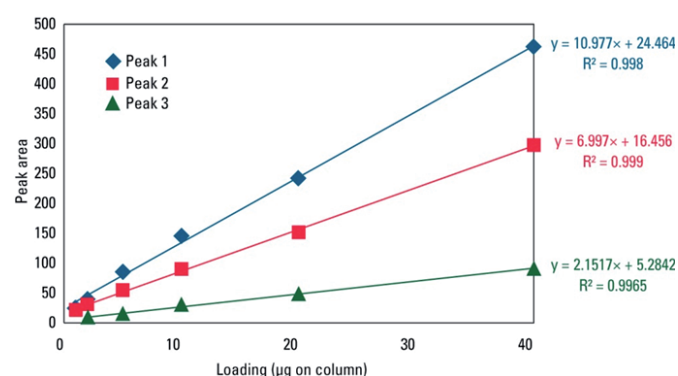| Column:	TSKgel FcR-IIA-NPR, 5 um, 4.6 mm ID × 7.5 cm L
Mobile phase:	A: 50 mmol/L citrate +150 mmol/L NaCl, pH 6.5
	B: 50 mmol/L citrate +150 mmol/L NaCl, pH 4.5
Gradient:	0-100% B (2-20 min)
Flow rate:	1 mL/min
Detection:	UV @ 280 nm
Sample:	CHO cell culture supernatant with therapeutic antibody

LINEARITY OF TSKgel FCR-IIIA-NPR COLUMN

$y = 10.977x + 24.464$
$R^2 = 0.998$

$y = 6.997x + 16.456$
$R^2 = 0.999$

$y = 2.1517x + 5.2842$
$R^2 = 0.9965$

➤ Figure 1

Column:	TSKgel FcR-IIA-NPR, 5 um, 4.6 mm ID × 7.5 cm L
Mobile phase:	A: 50 mmol/L citrate, pH 6.5
	B: 50 mmol/L citrate, pH 4.5
Flow rate:	1 mL/min
Detection:	UV @ 280 nm
Sample:	Trastuzumab