TSKgel FcR-IIIA-NPR

Fc Receptor Tips:

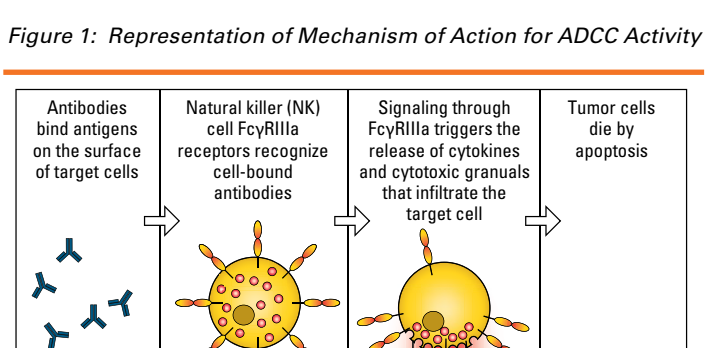
- The TSKgel FcR-IIIA-NPR column is offered in PEEK hardware to promote low adsorption of biomolecules. The ligand is bonded to a nonporous stationary phase allowing high throughput affinity chromatography at the analytical scale.
- As with all columns used with gradient elution, affinity columns should be washed with final elution buffer prior to their re-equilibration with initial (binding) buffer. Always wash the column and the LC system with halide-free buffer at the end of the day.
- Column shipping solvent is an aqueous solution of 10 mmol/L citrate buffer, pH 6.5, with 0.025% ProClin300.
- The TSKgel FcR-IIIA-NPR column is supplied with an Inspection Data Sheet, which includes a QC chromatogram, an Analysis Report, which includes gel batch data, and an OCS Sheet summarizing the recommended operating conditions for optimum column performance.
- A separate TSKgel Column Instruction Manual that reviews general guidelines for column installation and care, as well as troubleshooting tips for commonly encountered problems, can be downloaded from the Tosoh Bioscience LLC website (www.tosohbioscience.com).

About: Fc Receptor Affinity Chromatography

Monoclonal antibodies (mAbs) comprise the largest class of glycosylated protein therapeutics currently on the market and glycosylation is known to be a major source of mAb heterogeneity[1]. N-glycosylation of IgG-Fc of mAbs is known to impact drug therapeutic mechanism of action (MOA), thus monitoring glycan critical quality attributes (CQAs) is essential for maintaining drug product safety and efficacy[2-5].

Antibody-dependent cell mediated cytotoxicity (ADCC) has been recognized as a therapeutic MOA for several mAbs. ADCC begins when the Fab region of an antibody binds to an antigen on a target cell and the Fc domain binds Fcγ receptors on the surface of an effector cell. Signaling through the Fcγ receptor triggers degranulation into a lytic synapse which ultimately leads to apoptosis (Figure 1). In particular, FcγRIIIa expressed on peripheral blood mononuclear cells (PBMC) or natural killer (NK) cells have been shown to play an essential role in ADCC[6].

Figure 1: Representation of Mechanism of Action for ADCC Activity



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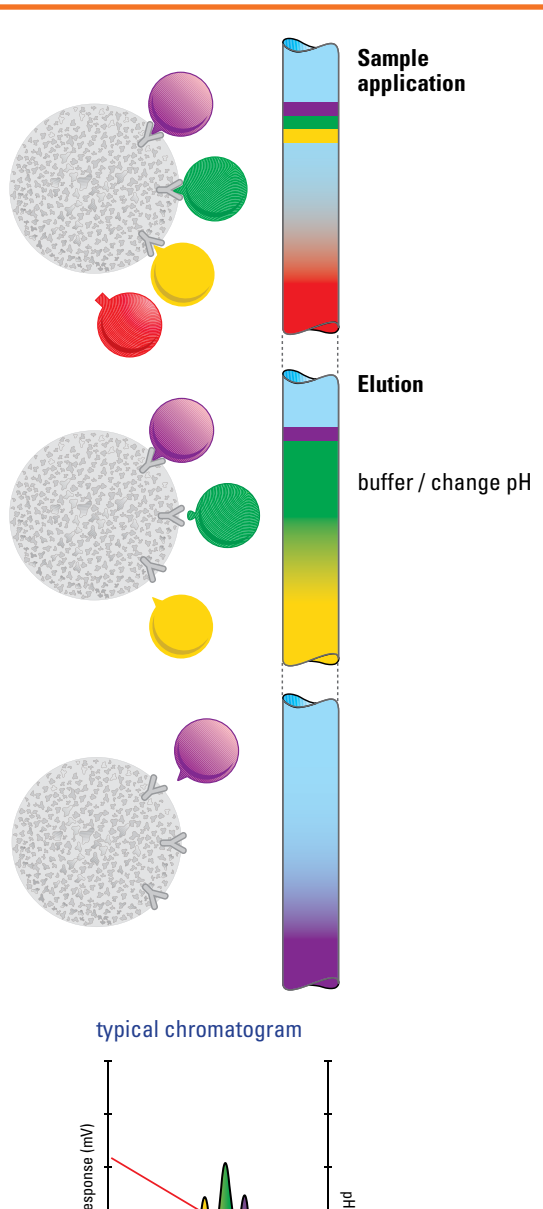
In FcγRIIIa chromatography, purified drug product or crude feed stock is passed through a column under conditions that promote binding of N-glycosylated mAbs to the immobilized recombinant FcγRIIIa ligand on the surface of the particle. mAbs which do not possess glycosylation elute in the void volume. Elution of mAb glycoforms present in the sample is performed by altering the pH of the mobile phase in order to disrupt the target/ligand interactions. Glycoforms are eluted as multiple peaks, correlating to the affinity of the N-glycosylation of IgG-Fc for the recombinant FcγRIIIa ligand (Figure 2).

Figure 2: FcγRIIIa Affinity Chromatography



About: TSKgel FcR-IIIA-NPR Affinity Chromatography Column

TSKgel FcR-IIIA-NPR is a 5 µm, 4.6 mm ID x 7.5 cm PEEK column for high performance affinity chromatography. This column is designed for the separation of mAb efficacy variants on the basis of affinity of the N-linked glycosylation in the Fc Region of IgG_1-Fc for the recombinant FcγRIIIa stationary phase. The ligand is bonded to nonporous polymethrylate beads, providing efficient and rapid separation of mAb glycoforms. The rugged nature of the column facilitates analysis both prior to or after purification.

TSKgel FcR-IIIA-NPR can be utilized for the following applications:

- Comparison between biosimilar/biobetter and innovator reference product
- QC Analysis of lot-to-lot difference for mAb drug products
- Monitoring fermentation stage of cell culture media
- Screening the potential of cell lines for ADCC activity

Attributes

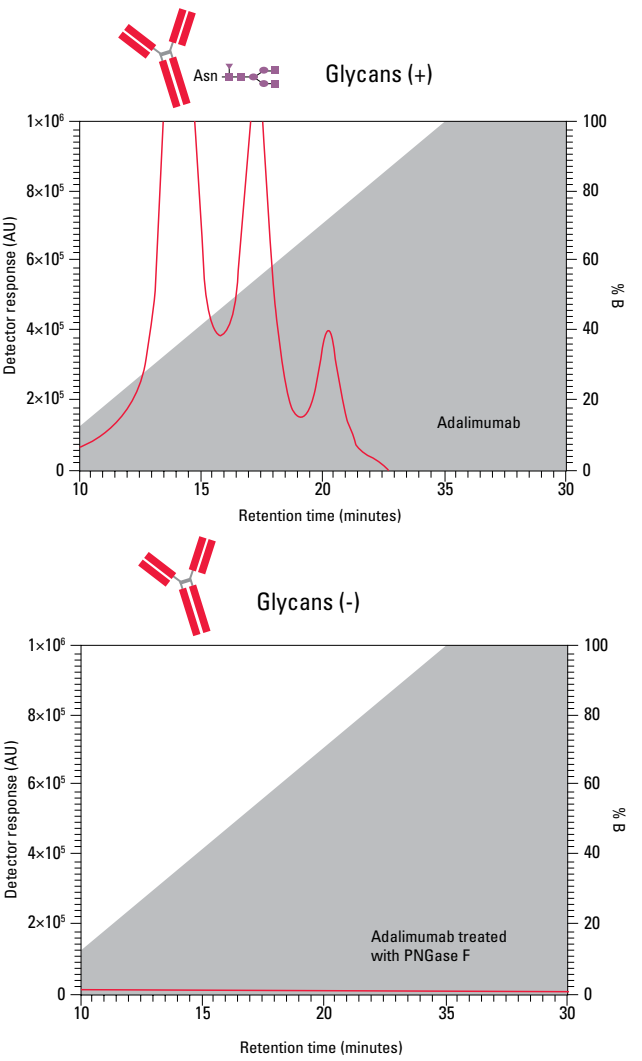
Table 1: Product Attributes

Ligand	Modified recombinant FcγRIIIA (*E. Coli* expression system, non-glycosylated)
Base matrix	Nonporous resin, 5 µm
Column	4.6 mm ID x 7.5 cm, PEEK
Sample mass	5 – 50 µg of IgG (recommended)
Flow rate	Max 1.0 mL/min
Recommended temperature	15 °C ~ 25 °C (column oven)
pH stability	pH 4 - 8 (short term) pH 5 - 7 (long term)
Recommended buffer system	A: 50 mmol/L citrate buffer, 150 mmol/L NaCl, pH6.5 B: 50 mmol/L citrate buffer, 150 mmol/L NaCl, pH4.5
Maximum pressure	9 MPa

Affinity for N-glycosylated mAbs

Figure 3 shows the specificity of the recombinant FcγRIIIA ligand for mAbs which contain N-glycans. When adalimumab is injected onto the column, three peaks are able to be resolved, corresponding with the molecule's glycan heterogeneity. Treatment of adalimumab with PNGase F results in the de-glycosylation of the sample. Upon injection of the de-glycosylated sample onto TSKgel FcR-IIIA-NPR, the sample is not retained. These results show the affinity of the FcγRIIIA ligand for mAb glycoforms.

Figure 3: HPLC Analysis of adalimumab with and without PNGase F treatment using TSKgel FcR-IIIA-NPR

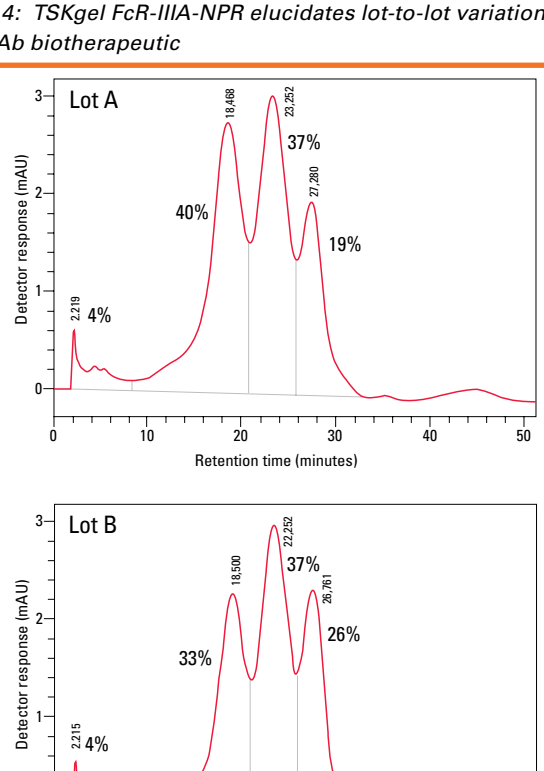


Column:	**TSKgel FcR-IIIA-NPR**
Mobile phase A:	50 mmol/L citrate, pH 6.5
Mobile phase B:	50 mmol/L citrate, pH 4.5
Flow rate:	1 mL/min
Detection:	UV @ 280 nm
Sample:	50 µL of adalimumab or PNGase F treated adalimumab (1 µg/µL)

mAb Quality Control

Figure 4 shows the utility of the TSKgel FcR-IIIA-NPR column for mAb quality control. Two lots of the same monoclonal antibody-based biotherapeutic were injected onto the column for analysis. Differences in relative peak area percentages indicate that lot-to-lot variations are present. This column can provide a fast and effective way to detect differences in mAb glycoform prevalence in drug product.

Figure 4: TSKgel FcR-IIIA-NPR elucidates lot-to-lot variation of a mAb biotherapeutic

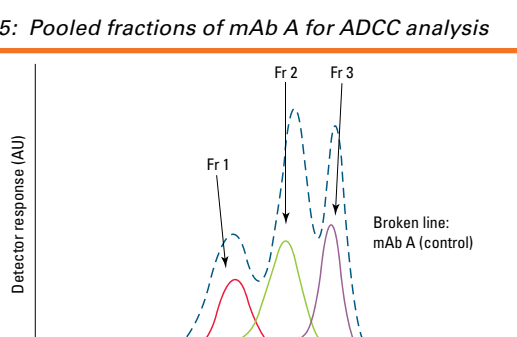


Column:	**TSKgel FcR-IIIA-NPR**
Mobile phase A:	50 mmol/L citrate, pH 6.5
Mobile phase B:	50 mmol/L citrate, pH 4.5
Flow rate:	1 mL/min
Detection:	UV @ 280 nm
Sample:	mAb based biotherapeutic, Lot A and B

ADCC Efficacy

Affinity of a mAb glycoform for FcγRIIIa is correlated to increased ADCC activity. Peak fractions from a typical separation of mAb A were collected and pooled as shown in Figure 5. Figure 6 shows the corresponding ADCC activity of each sample. As indicated, the most retentive component, fraction 3, displays the highest level of ADCC activity. Each individual fraction shows a different level of ADCC activity than calculated for the unfractioned mAb sample. The TSKgel FcR-IIIA-NPR column allows the generation of additional insight regarding ADCC activity than can be identified by analyzing the mAb alone.

Figure 5: Pooled fractions of mAb A for ADCC analysis

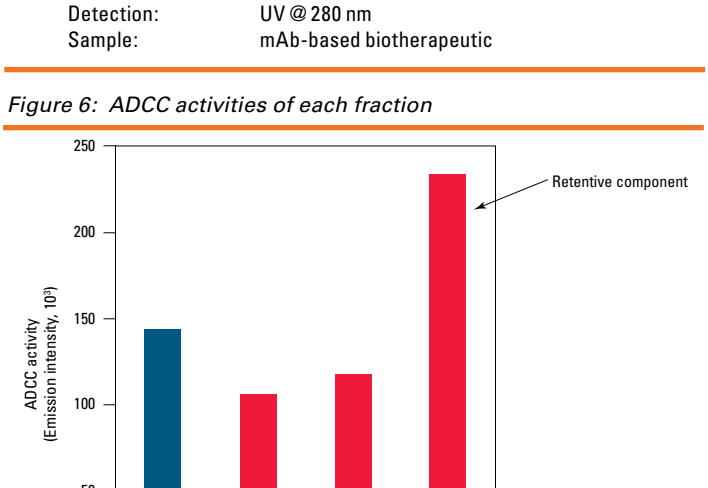


Content (%)

	Peak 1	Peak 2	Peak 3
Control	22	44	34
Fr 1	83	17	0
Fr 2	12	80	8
Fr 3	0	9	91

Column:	**TSKgel FcR-IIIA-NPR**
Mobile phase A:	50 mmol/L citrate, pH 6.5
Mobile phase B:	50 mmol/L citrate, pH 4.5
Flow rate:	1 mL/min
Detection:	UV @ 280 nm
Sample:	mAb-based biotherapeutic

Figure 6: ADCC activities of each fraction



Ordering Information

Part #	Description	Matrix	Housing	ID (mm)	Lenght (cm)
23513	TSKgel FcR-IIIA-NPR	Polymer	PEEK	4.6	7.5

References

1. Ecker et al; *mAbs*; 2015, 7, 9-14
2. de Val and Kontoravdi; *Biotechnol Prog*; 2010, 26, 1505-1527
3. Arnold et al; *Annu Rev Immunol*; 2007, 25, 21-50
4. Vidarsson et al; *Front Immunol*; 2014, 5, 520
5. Kiyoshi et al; *Nature/Scientific Reports*; 2018, 8, 3955
6. Shields et al; *J Biol Chem*; 2001, 276, 6591-6604