TOSOH

Product Overview

TSKgel® FcR-IIIA-NPR Affinity Column

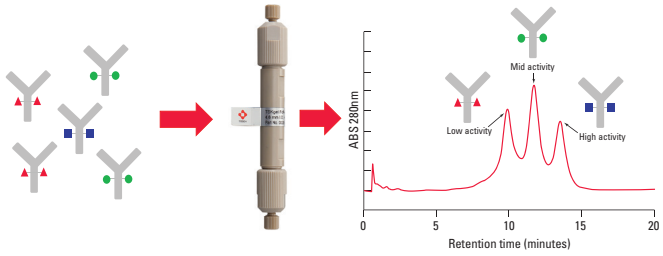
Providing fast evaluation of ADCC activity

TSKgel FcR-IIIA-NPR 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.

Introduction

Fc gamma IIIa receptor plays a key role in antibody-dependent cell-mediated cytotoxicity (ADCC). ADCC is a crucial mechanism of action (MoA) of anti-tumor therapeutic antibodies. The Fc-glycans of antibodies are known to play an important role in Fc-mediated effector functions. Hence, separation patterns of therapeutic antibodies on TSKgel FcR-IIIA-NPR can be correlated to Fc N-glycans. Terminal galactose residues increase affinity to FcR gamma while core fucose residues reduce it. This correlates with the known influence of galactose and fucose on ADCC activity. Accordingly, early eluting peaks of TSKgel FcR-IIIA-NPR represent glycoforms with low ADCC activity while late eluting peaks represent glycoforms with high ADCC activity *(Figure 1)*.

Figure 1. Separation of mAb glycoforms according to their affinity to FC receptor/ADCC activity



A rapid thirty minute separation allows the analysis of large numbers of mAb samples to gain valuable initial information on the distribution of glycoforms and expected ADCC activity. This initial and efficient method can be applied to purified samples and supernatant alike and can therefore be used in many phases of development and production 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.

Highlights

- Easy and reproducible HPLC analysis based on FcγIIIa receptor affinity of mAbs
- Unique glycoprotein elution profile of IgG allows assessment of ADCC activity
- Applicable to purified samples and cell culture supernatant alike
- Fast cell line screening, upstream development, lot-to-lot comparison

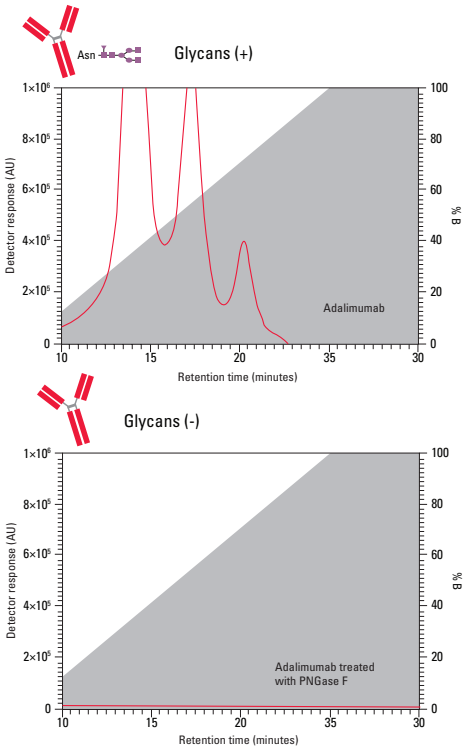
TSKgel FCR-IIIA-NPR Properties

TSKgel FcR-IIIA-NPR is a 5 µm, 4.6 mm ID x 7.5 cm analytical column for high performance affinity chromatography. The stationary phase utilizes a non-glycosylated, recombinant, human FcγRIIIa protein bound to a non-porous polymer bead and is packed in PEEK hardware. This ligand was engineered to have inherently high stability and nearly identical selectivity to wild type FcγIIIa receptor. The column separates IgG glycoforms based on their ADCC activity, allowing for fast assessment of biologic activity.

Separation of mAb Glycoforms

Figure 2 demonstrates the specificity of the recombinant FcγRIIIA ligand for N-glycans of the Fc domain of mAbs. Adalimumab analyzed with TSKgel FcR-IIIA-NPR shows a typical pattern of three peaks, corresponding with the molecule's glycan heterogeneity. Treatment of adalimumab with PNGase F deglycosylates the sample. Deglycosylated adalimumab does not bind to TSKgel FcR-IIIA-NPR; the N-glycan related peaks are absent. These results show the affinity of the FcγRIIIA ligand for mAb glycoforms.

Figure 2. Analysis of adalimumab and deglycosylated adalimumab

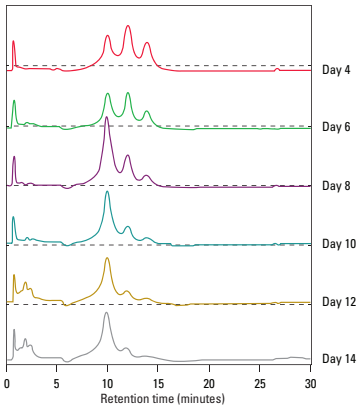


Column:	TSKgel FcR-IIIA-NPR
Mobile phase A:	50 mmol/L citrate, pH 6.5
Mobile phase B:	50 mmol/L citrate, pH 4.5
Flow rate:	1 mL/min
Detection:	UV @ 280 nm
Sample:	50 µL of adalimumab or PNGase F treated adalimumab (1 µg/µL)

Upstream Monitoring of CHO Cell Culture

TSKgel FcR-IIIA-NPR can be applied to analyze monoclonal antibodies directly from cell culture supernatant. This can be used either for fast cell line screening in early R&D, for upstream optimization or for upstream monitoring, as shown in *Figure 3*. CHO cell culture supernatant was sampled periodically, filtered, purified by protein A capturing, and injected on TSKgel FcR-IIIA-NPR. Monoclonal antibody glycoform patterns changed towards lower ADCC activity variants during the course of the culture.

Figure 3. Cell culture monitoring

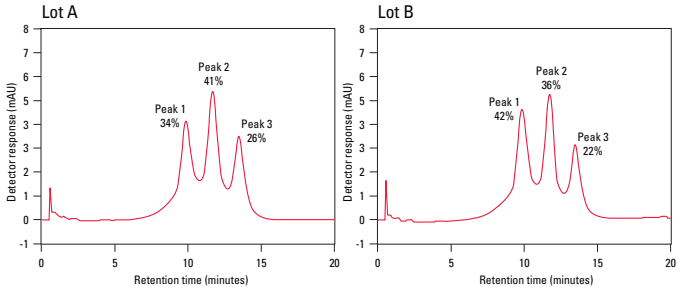


CHO cell culture was kindly provided by Manufacturing Technology Association of Biologics.

Lot-to-Lot Comparison of mAbs

Figure 4 illustrates a comparison of two production lots of a therapeutic antibody. Lot B shows a higher percentage of glycoforms with lower ADCC activity than lot A.

Figure 4. HPLC analysis of two lots of a therapeutic antibody



Ordering Information

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