TSKgel FcR-IIIA-NPR AFFINITY COLUMN FOR 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

Fcγ 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).

A rapid thirty-minute separation allows the analysis of large numbers of mAb samples to gain valuable first information on the distribution of glycoforms and expected ADCC activity. This fast 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 FcγRIIIa 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.

ANALYSIS 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.

ANALYSIS OF mAb GLYCOFORMS ACCORDING TO THEIR AFFINITY TO FC RECEPTOR / ADCC ACTIVITY

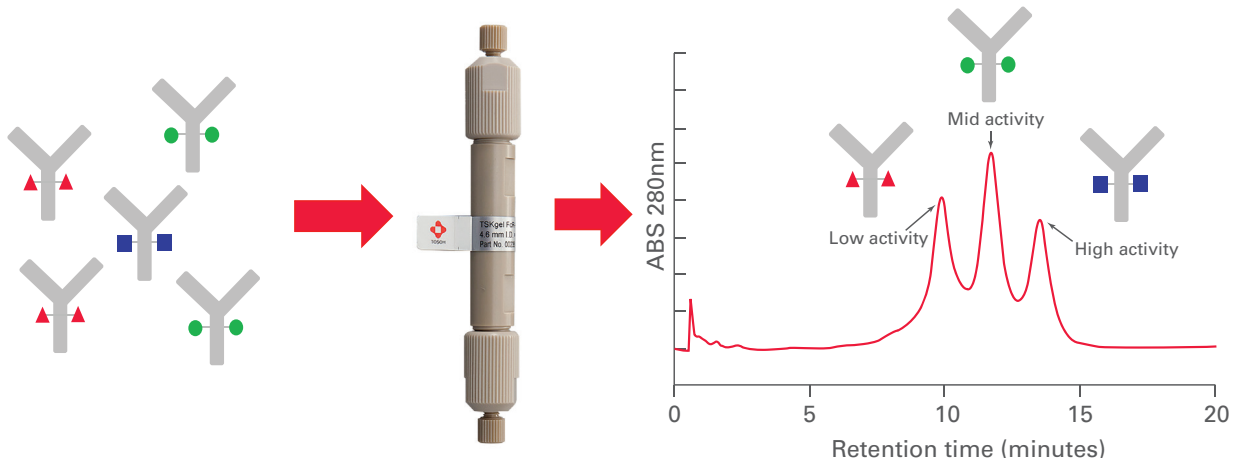

Figure 1

Treatment of adalimumab with PNGase F de-glycosylates the sample. De-glycosylated 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.

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. mAb glycoform patterns changed towards lower ADCC activity variants during the course of the culture.

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.

ANALYSIS OF ADALIMUMAB AND DE-GLYCOSYLATED ADALIMUMAB

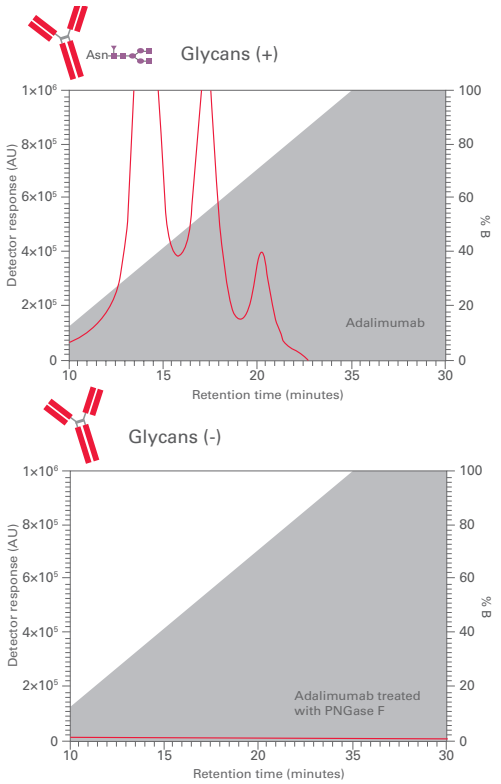

Figure 2

Column: TSKgel FcR-IIIA-NPR; Mobile Phase: A: 50 mmol/L citrate, pH 6.5; B: 50 mmol/L citrate, pH 4.5; Flow Rate: 1 mL/min; Temp: 25 °C; Detection: UV @ 280 nm, Sample: 50 µL of adalimumab or PNGase F treated adalimumab (1 µg/µL); Data: master thesis Leila Ghaleh, TU Darmstadt

CELL CULTURE MONITORING

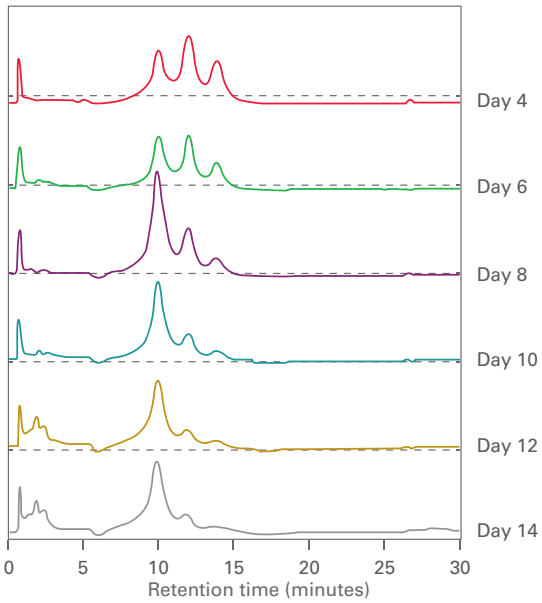

Figure 3

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

HPLC ANALYSIS OF TWO LOTS OF A THERAPEUTIC ANTIBODY

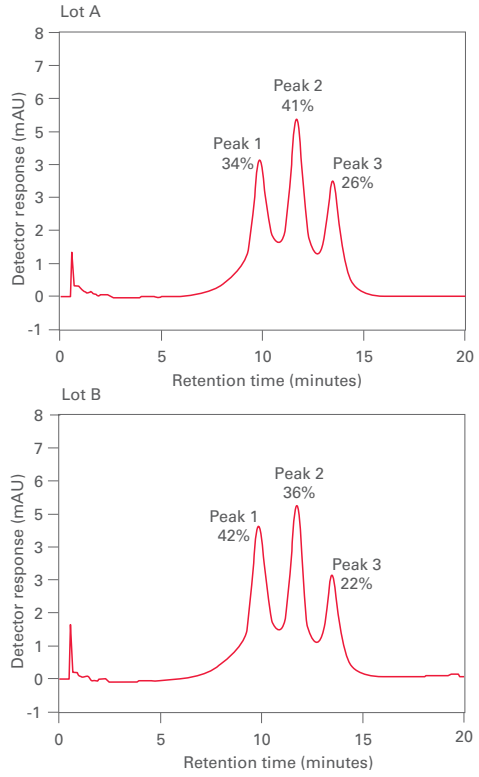

Figure 4

Ordering information

TSKgel FcR-IIIA-NPR

Part-No	Description	Matrix	Housing	Dimensions
0023513	TSKgel FcR-IIIA-NPR	NPR, polymer	PEEK	4.6 mm ID x 7.5 cm L

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