TOSOH

Product Overview

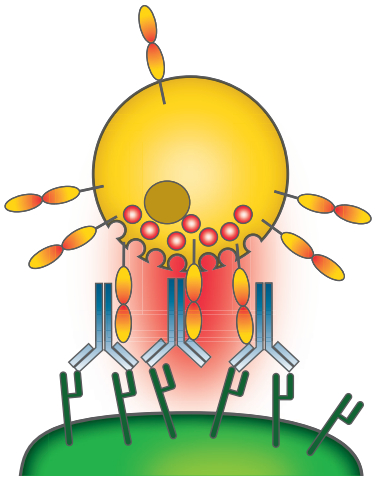
FcR-IIIA Affinity Chromatography – The Elegant Way to Optimize and Analyze Antibodies

The two TSKgel® columns, FcR-IIIA-NPR and FcR-IIIA-5PW, separate antibodies based on the affinity of their Fc region for a particular Fc receptor (FcγIIIa). The affinity has a direct influence on the mode of action of an antibody. Therefore, the columns are a useful tool for the analysis and characterization of antibodies and can be applied to optimize and control their production and storage conditions.

FcγIIIa receptor affinity: A key element in immune resonse

The FcγIIIa receptor is typically expressed on natural killer immune cells. As the name indicates, the receptor binds Fc regions of antibodies as well as of antibodies used as treatment for diseases. Binding of the FcγIIIa receptor to the Fc-part of an antibody stimulates the natural killer cells to release cytotoxic factors to induce cell death. As the antibody links the natural killer cell (via its Fc region) to a pathogenic cell (via its antigen binding Fab region), the pathogenic cell will be killed. The process is called antibody-dependent cellular cytotoxicity (ADCC) and is required for many cancer-targeting therapeutic antibodies to be efficient *(Figure 1)*.

Figure 1. Antibody-dependent cell-mediatated cytotoxicty (ADCC)



The FcγIIIa receptor on natural killer cells (orange) is linked to pathogenic cells (green) by the Fc part of an antibody (blue). Binding of the Fc part leads to release of cytotoxic factors and induces cell death in the target cell. *(Original image by Satchmo2000, distributed under a CC-BY 3.0 license)*

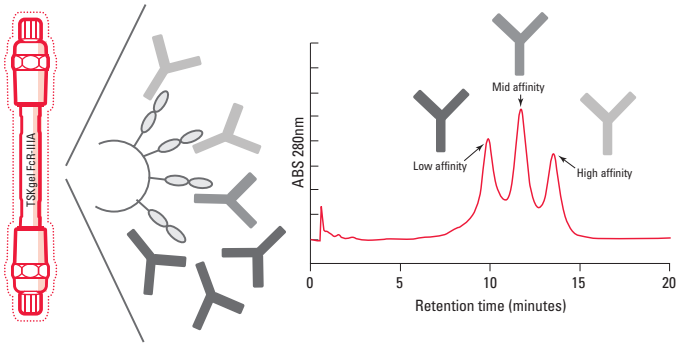
Using the FcγIIIa receptor as a ligand for chromatography

Due to its importance for therapeutic antibodies, Tosoh created an FcγIIIa receptor mimicking the receptor of natural killer cells and coupled it to the stationary phase of HPLC columns. This way the affinity of antibody samples to the FcγIIIa receptor can be analyzed and isolation of antibodies with different affinities is possible *(Figure 2)*.

Short profile of the TSKgel FcR-IIIA columns

Ligand:	recombinant FcγIIIa receptor *(E.coli)*
Separation mechanism:	affinity of antibody Fc region to FcγIIIa receptor
Elution:	decreasing pH gradient
Base material:	polymethacrylate
Hardware:	PEEK
Versions:	**TSKgel FcR-IIIA-NPR** for quick analyses, cell-line screenings, lot-to-lot comparisons **TSKgel FcR-IIIA-5PW** for collecting fractions with different FcR affinities to characterize them

Figure 2. Separation principle of TSKgel FcR-IIIA columns



The TSKgel FcR-IIIA affinity columns employ a recombinant version of the FcγIIIa receptor to separate antibodies according to their affinity to the receptor (left). Elution occurs with a decreasing pH gradient while affinity is increasing with lower elution pH (right).

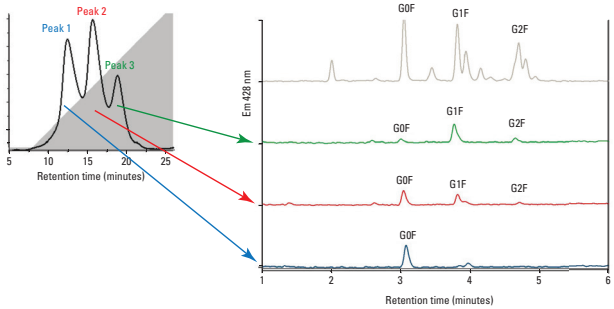
Isolate antibodies according to FcR affinity from a hetrogeneous sample (TSKgel FcR-IIIA-5PW)

Expression of antibodies typically leads to an antibody mixture with different affinities to the FcγIIIa receptor. The TSKgel FcR-IIIA-5PW column can be loaded with ~5 mg of antibody sample and resolves three affinity fractions. These can be employed to analyze structural and functional differences linked to differences in FcR affinity.

Correlate glycan structure to FcR affinity

Glycosylation of an antibody Fc region is closely correlated with its affinity to the FcγIIIa receptor. Fractions of low, mid and high affinity antibodies can be collected and analyzed by HILIC or MS to determine differences in glycosylation patterns *(Figure 3)*.

Figure 3. Glycan pattern analysis by HILIC in three FcR affinity fractions

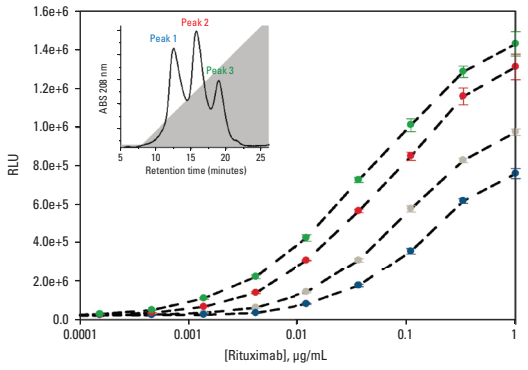


The TSKgel FcR-IIIA-5PW column can be applied to determine glycosylation differences in antibody fractions with different FcR affinities

Correlate ADCC activity to FcR affinity

The affinity of an antibody Fc region to the FcγIIIa receptor is important as it regulates the cell cytotoxic activity and thus efficacy of antibodies acting via ADCC. The relation can be determined using fractions of differing FcR affinities and cell-based ADCC assays *(Figure 4)*.

Figure 4. ADCC activity of three FcR affinity fractions



The TSKgel FcR-IIIA-5PW column can be applied to measure the ADCC activity in antibody fractions with different FcR affinities. Grey represents Rituximab; the other colors refer to fractions of the three peaks as indicated.

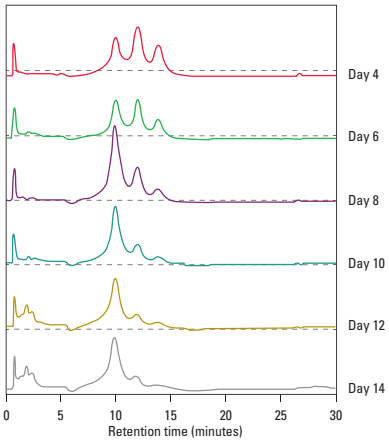
Rapidly analize FcR affinity differences of antibodies (TSKgel FcR-IIIA-NPR)

Up to now, FcR affinity can be determined indirectly by glyco-profiling or ADCC assays, or directly which requires dedicated instrumentation, time and tedious sample preparation. In contrast, the TSKgel FcR-IIIA-NPR column analyzes FcR affinities of antibody samples in 20-30 minutes directly in cell culture supernatant.

Screen production conditions

20-30 minutes of analysis using the TSKgel FcR-IIIA-NPR column quickly shows how changing upstream conditions can impact FcR affinity *(Figure 5)*.

Figure 5. Screen conditions to result in optimal FcR affinity properties



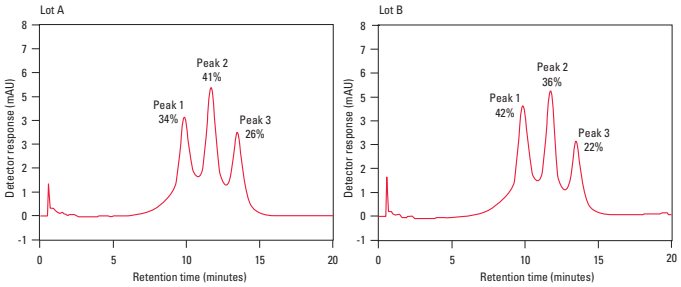
CHO cell culture was kindly provided by Manufacturing Technology Association of Biologics.

The TSKgel FcR-IIIA-NPR column quickly screens antibody samples regarding their FcR affinity. Shown are chromatograms of product after the indicated fermentation time. *CHO cell culture was kindly provided by Manufacturing Technology Association of Biologics.*

Compare antibodies

Testing of antibody products is necessary to determine lot-to-lot variations and stability after storage. Chromatography using FcR-IIIA can quickly and easily discern differences in antibody batches for additional quality control or compare originators to biosimilars *(Figure 6)*.

Figure 6. HPLC analysis of two lots of a therapeutic antibody

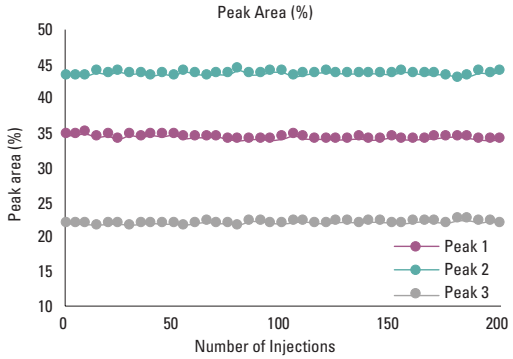


Lot-to-lot variations are easily detected with the TSKgel FcR-IIIA-NPR column

TSKgel FcR-IIIA-NPR stable for > 200 injections

The TSKgel FcR-IIIA columns employ a recombinant FcγIIIa receptor protein as ligand. Durability of the TSKgel FcR-IIIA-NPR column and ligand was tested by analyzing the stability of the results using Rituximab as a sample. Determined was the composition of the antibody with the three affinity peaks as shown by the % of total area. *Figure 7* shows the column provided stable results over 200 injections.

Figure 7. Stability of the repetitive analysis of Rituximab



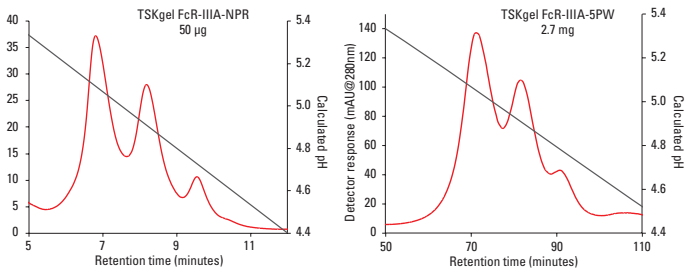
Repetitive injection of 25 µg Rituximab on TSKgel FcR-IIIA-NPR. The proportion of the three affinity peaks (low, medium, high) in the total area is shown and proves stability over 200 injections.

How the two TSKgel FcR-IIIA columns compare

The TSKgel FcR-IIIA-NPR and the FcR-IIIA-5PW share the same ligand and separation mechanisms. This is confirmed by the pH at which the three affinity fractions elute: injecting the same sample onto each column results in the identical elution pH of each peak *(Figure 8)*.

The semi-preparative TSKgel FcR-IIIA-5PW column and the analytical TSKgel FcR-IIIA-NPR column differ in loading capacity and run times. Whereas the NPR column employs non-porous particles to allow fast analysis of low protein amounts, the 5PW consists of a higher volume and fully porous particles to increase the interaction surface and loading capacity *(Figure 8)*. This makes it possible to collect fractions of sufficient amount of protein to use it for further characterizations. These include cell-based ADCC assays, HILIC-based glycosylation analysis or determination of binding kinetics.

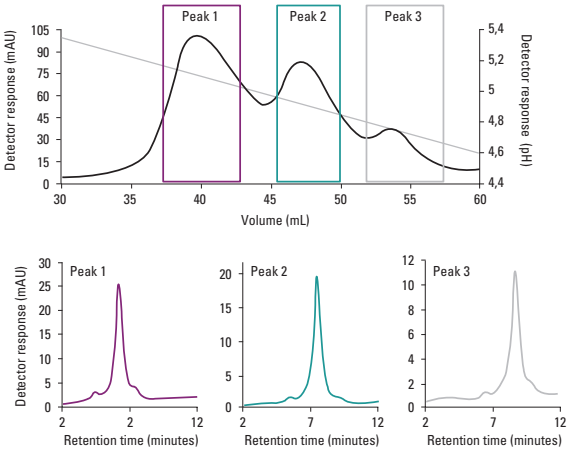
Figure 8. Trastuzumab separation on analytical (NPR) and semi-preparative (5PW) column



Injection of a Trastuzumab onto TSKgel FcR-IIIA-NPR and -5PW columns. Elution pH of each peak is comparable on both columns.

Fractions collected through FcR affinity chromatography contain predominantly a homogenous affinity profile as can be confirmed by analysis with the TSKgel FcR-IIIA-NPR column *(Figure 9)*.

Figure 9. FcR affinity fractions of Trastuzumab analyzed on TSKgel FcR-IIIA-NPR



5 mg of Trastuzumab injected onto TSKgel FcR-IIIA-5PW (upper figure) and fractions of each peak analyzed on TSKgel FcR-IIIA-NPR (lower figures).

Ordering Information

Part #	Description	Ligand	Porous	Matrix	Particle size	Dimension (ID x cm)	Pressure limit	Sample size
23513	TSKgel FcR-IIIA-NPR	Recombinant FcγIIIa receptor	No	Polymethacrylate	5 µm	4.6 × 7.5	9 MPa	~50 µg
23532	TSKgel FcR-IIIA-5PW	Recombinant FcγIIIa receptor	Yes	Polymethacrylate	10 µm	7.8 × 7.5	1 MPa	1-5 mg

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