



# Correlation of FcR Affinity Chromatography with Glycan Pattern and ADCC Activity of a Therapeutic Antibody

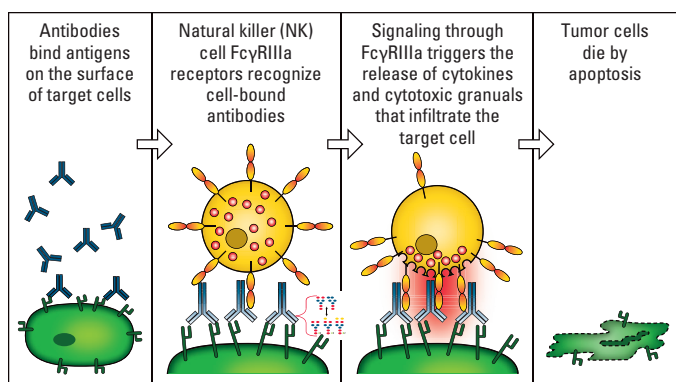
## Introduction

Antibody-dependent cell-mediated cytotoxicity (ADCC) is an important mechanism of action (MOA) of monoclonal antibodies used in cancer treatment. Selecting suitable cell lines and optimizing culture conditions towards expression of antibody candidates with desired ADCC activity is an essential part of the R&D process. A fast and straightforward approach to easily access ADCC activity would facilitate screening of a large number of clones or monitoring the effect of upstream process variations. Other stages of R&D and production could benefit from fast ADCC assessment as well: comparing biosimilar and originator, detecting lot-to-lot variations, monitoring product stability, to name but a few.

## Fc Receptor and ADCC Activity

ADCC starts with the binding of the Fab region of an antibody to a target cell, e.g. a cancer cell. Binding of the Fc domain of that antibody to Fc $\gamma$  receptors on the outer membrane of natural killer (NK) cells triggers degranulation into a lytic synapse and finally the apoptosis of the cancer cell (Figure 1). The glycan microheterogeneity of the Fc domain, in particular on the galactose and core-fucose levels<sup>1</sup>, influences binding of the Fc domain to Fc $\gamma$  receptors.

Figure 1. Antibody-dependent cell-mediated cytotoxicity

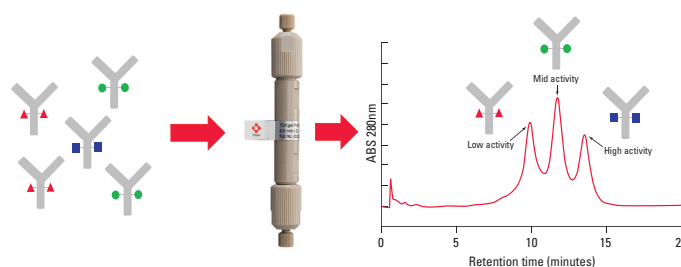


Original image by Satchmo2000, distributed under a CC-BY 3.0 license.

Current ADCC activity tests are either cell based bioassays or surface plasmon resonance (SPR) measurements using immobilized Fc $\gamma$  receptor. A new approach combines the specificity of the Fc $\gamma$ IIIa receptor (Fc $\gamma$ RIIIa) with the easy handling of an HPLC method.

For Fc receptor affinity chromatography, a recombinant Fc $\gamma$ IIIa receptor ligand is immobilized on a stationary phase. Glycoforms of an antibody sample can be partly separated based on the strength of their binding to the FcR ligand. Resulting peaks can be assigned to low, medium and high ADCC activity (Figure 2).

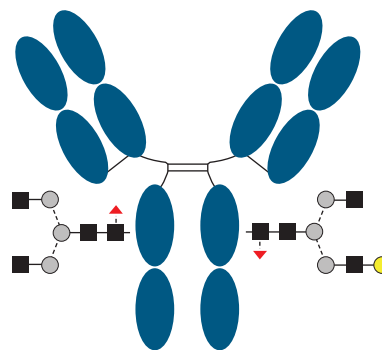
Figure 2. Fc $\gamma$ RIIIa affinity chromatography



Taking the well-known therapeutic antibody rituximab as an example, this application note demonstrates that the pattern of Fc $\gamma$ RIIIa affinity chromatography shows a good correlation with the results obtained by an ADCC reporter assay. Fractions collected from HPLC peaks with different receptor affinity also show different glycosylation patterns at the Fc domain.

Rituximab (Figure 3) is a recombinant chimeric human/mouse monoclonal IgG<sub>1</sub> antibody approved in 1997 and used to treat certain autoimmune diseases and types of cancer. Besides other effects of rituximab, its Fc portion mediates antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC)<sup>2</sup>. N-glycans bound to the Fc domain of rituximab contain mainly G0F and G1F structures.

Figure 3. Rituximab

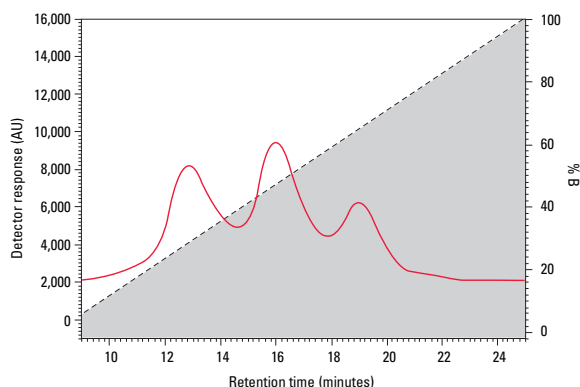


## FcR Affinity Chromatography of Rituximab

In Fc $\gamma$ RIIIa affinity chromatography, purified antibody or cell culture supernatant is injected under conditions that promote binding of monoclonal antibodies (mAbs) to the Fc $\gamma$ RIIIa ligand. Elution of bound mAb variants is performed by lowering the pH of the mobile phase in order to disrupt the target/ligand interactions. The higher the affinity of a mAb variant to the receptor, the higher the retention time of the respective peak.

FcR affinity chromatography analysis of rituximab on the new TSKgel<sup>®</sup> FcR-IIIa-NPR column results in three peaks representing variants with low, medium and high Fc $\gamma$ RIIIa affinity (Figure 4). Fractions out of the three peaks were collected. For each peak, cleaved and 2AB-labeled N-glycans were characterized by HILIC-UHPLC and ADCC activity was analyzed with a standard ADCC reporter bioassay kit (Promega).

Figure 4. Fc $\gamma$ R affinity analysis of rituximab on TSKgel FcR-IIIa-NPR



## ADCC Bioassay of Rituximab and FcR Affinity Fractions

The Fc effector reporter bioassay uses the Fc $\gamma$ R and NFAT-mediated activation of luciferase activity in effector cells to determine ADCC efficacy and potency of antibodies. Figure 5 shows the ADCC reporter bioassay response to rituximab and to the three fractions collected from FcR affinity chromatography (low, medium, high Fc $\gamma$ R affinity). Rituximab is shown as the grey points, fraction 1, representing low Fc $\gamma$ R affinity is shown in blue, fraction 2 representing medium Fc $\gamma$ R affinity is shown in red, and fraction 3 representing high Fc $\gamma$ R affinity is shown in green<sup>3</sup>.

Figure 5. ADCC reporter bioassay response to rituximab

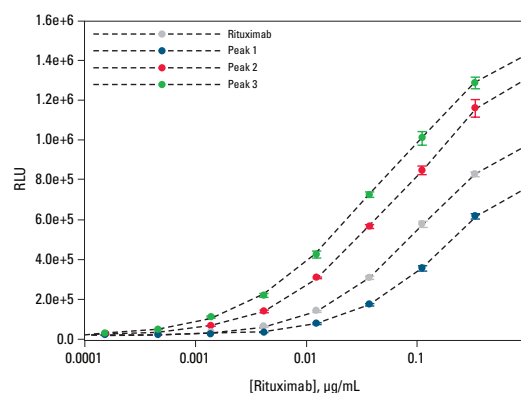


Table 1 shows the EC<sub>50</sub> values obtained by the reporter bioassay test. The lower the EC<sub>50</sub> value, the higher the ADCC potency. As expected, peak three (high Fc $\gamma$ R affinity, green) shows the highest ADCC potency and efficacy in the bioassay. Peak two shows the intermediate and peak 1 shows the lowest ADCC efficacy and potency. ADCC efficacy and potency of the original rituximab lies between the low and medium affinity fractions.

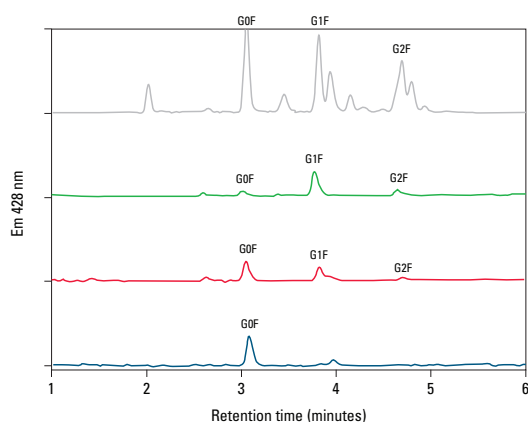
Table 1. The dose-response curve of ADCC data was fitted with a 4-parameter model using a sigma plot

EC <sub>50</sub> values obtained by the reporter bioassay test	
Antibody	EC <sub>50</sub> (µg/mL)
Rituximab	0.098
Peak 1	0.153
Peak 2	0.072
Peak 3	0.049

## Glycan Analysis of FcR Affinity Fractions

The glycan profile of the collected fractions was analyzed by hydrophilic interaction chromatography (HILIC). *Figure 6* shows the glycan pattern of the FcR affinity fractions compared to a glycan library. The antibody glycoforms collected in peak 3 (highest affinity) show mainly galactose containing N-glycans (G1F and G2F). Peak 2 glycoforms contain more G0F glycans than peak 3 and glycoforms collected in peak 1 (lowest affinity) show predominantly fucosylated glycans without galactose units (G0F).

**Figure 6.** HILIC analysis of oligosaccharides of the three FcR affinity fractions



HILIC analysis of oligosaccharides of the three FcR affinity fractions (Peak 1 blue, Peak 2 red, Peak 3 green) compared with a 2-AB labeled biantennary glycan library (grey)<sup>3</sup>.

## Conclusions

The ADCC activity bioassay results show that high retention on the TSKgel FcR-IIIa-NPR column corresponds to a high ADCC activity. The HILIC-UHPLC glycosylation pattern analysis of the FcR affinity fractions also matches the common understanding that terminal galactose units of Fc-glycans typically enhance affinity to FcγRIIIa and ADCC activity while core fucose units decrease ADCC activity of antibodies. These results confirm that FcγRIIIa affinity chromatography allows fast assessment of biologic activity and glycoform pattern of antibodies.

## References

- <sup>1</sup> D. Reusch and M. L. Tejada, *Glycobiology* 2015, 25 (12): 1325-1324; doi: 10.1093/glycob/cwv065
- <sup>2</sup> G.J. Weiner; *Semin Hematol.* 2010; 47 (2): 115-123; doi: 10.1053/j.seminhematol.2010.01.011
- <sup>3</sup> Master Thesis Leila Ghaleh, TU Darmstadt

TSKgel and Tosoh Bioscience are registered trademarks of Tosoh Corporation  
Rituximab kindly provided by Rentschler Biopharma