# N-Glycan Analysis of Antibody-Drug Conjugate (ADC) using EZGlyco® mAb-N Kit with 2-AB

## Introduction

Antibody-Drug Conjugate (ADC) is an emergent drug in which a small-molecule medicine (payload) is attached to an antibody via a linker and is considered an innovative approach in cancer therapy. Antibodies have glycans attached to them, and it is known that the structure and amount of glycans affect the physicochemical properties, antibody-dependent cellular cytotoxicity (ADCC), and antigen recognition properties of the antibody. Hence, glycan analysis is an important technology in manufacturing process development and quality control, since glycans can vary depending on the culture conditions of antibody-producing cells and differences in cell lines, etc. Glycan analysis is as important for ADCs as for conventional antibody drugs, and an efficient sample preparation method for analysis is required. In this application note, we introduce an example of ADC glycan analysis using the antibody N-type glycan analysis kit, EZGlyco® mAb-N Kit with 2-AB.

## Experimental Procedure

### ADC Samples

The following two commercially available ADCs were used:
Trastuzumab deruxtecan (MedChemExpress, HY-138298A)
Trastuzumab emtansine (HY-P9921)

### Glycan Preparation

An antibody N-glycan sample prep Kit "EZGlyco® mAb-N Kit with 2-AB" (Sumitomo Bakelite, BS-X4410) was used to prepare 2-AB labeled glycan sample according to the kit's protocol. The ADCs were diluted to 40 µg/600 µL with Antibody Capturing Buffer supplied with the kit, and the entire amount was applied to the Antibody Capturing Column, followed by protein denaturation, glycan release, 2-AB labeling, and clean-up in accordance with the protocol. (Figure 1)

### LC-MS analysis

Of approximately 50 µL of the obtained 2-AB labeled glycan sample solution, 1 µL was subjected to LC-MS measurement. The measurement conditions were as follows:

**LC: Nexera, Shimadzu Corporation**
Column: ACQUITY UPLC® Glycan BEH Amide, 1.7 µm (2.1 mm I.D. x 150 mm L.)
Temperature: 40 deg. C.
Detector: RF-20Axs (Ex.: 330 nm, Em.: 420 nm)
Mobile phase A: acetonitrile/water (40:60) containing 0.1% formic acid
Mobile phase B: acetonitrile/water (90:10) containing 0.1% formic acid
Gradient: 100% A (0 min) → 100% B (50 min)
Flow rate: 0.2 mL/min
Injection volume: 1 µL

**MS: LCMS-IT-TOF, Shimadzu Corporation**
Ionization mode: ESI negative ion mode

### Estimation of glycan composition

Glycan composition was estimated from the mass data (*m/z* value) of each LC peak obtained by MS measurement using the GlycoMod Tool[1] .


**Figure 1. Schematic representation of ADC glycan preparation using EZGlyco® mAb-N Kit with 2-AB**

## Results

HPLC charts and estimated glycan structures[2] of 2-AB-labeled glycans prepared using the EZGlyco® mAb-N Kit with 2-AB are shown (Figure 2). These glycan structures are consistent with those reported for Trastuzumab (IgG) N-type glycans[3] , confirming that the EZGlyco® mAb-N Kit with 2-AB can be applied to glycan analysis of ADCs without any protocol changes.

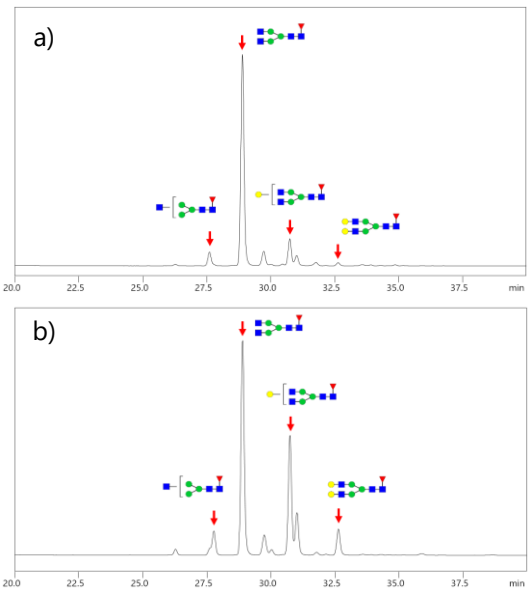


**Figure 2. ADC antibody N-glycan analysis results (HPLC)**
**a) Trastuzumab deruxtecan b) Trastuzumab emtansine**

## Conclusion

**EZGlyco® mAb-N Kit with 2-AB can powerfully support glycan analysis of antibody-drug conjugates (ADCs). It is expected to contribute to the efficiency of research and development and quality control of ADC, an emergent approach to cancer therapy.**

Reference

*1 Cooper C.A. et al, "GlycoMod - A software Tool for Determining Glycosylation Compositions from Mass Spectrometric Data" *Proteomics* 1:340-349 (2001).

*2 Examples of glycan structures estimated from the glycan composition by GlycoMod Tool and references.

*3 Segu, Z. et al. "A rapid method for relative quantification of N-glycans from a therapeutic monoclonal antibody during trastuzumab biosimilar development." *MAbs*. Vol. 12. No. 1. (2020)