Ludger Guide to Sialylation: I

Neu5Ac and Neu5Gc Quantitation

Sialic acid analysis is a regulatory requirement laid out in the ICH Q6B guidelines for characterisation of biopharmaceuticals. In particular, determination of relative levels of human and non-human type sialic acids (Neu5Ac/NANA and Neu5Gc/NGNA respectively) is important since some patients have high levels of anti-Neu5Gc antibodies which could lead to neutralization and rapid clearing of NeuGc-containing biopharmaceuticals (Nguyen et al, 2005). The significance of Neu5Ac and Neu5Gc when analysing glycoproteins means that it is often considered a critical quality attribute (CQA) for biotherapeutics.

A widely used method for determining the ratio of Neu5Ac to Neu5Gc is as follows:

- Release of sialic acid residues from the glycoprotein by mild acid hydrolysis.
- Fluorescent labeling of released sialic acids with1,2-diamino-4,5-methylenoxybenzene (DMB).
- Relative quantitative analysis of DMB-labelled sialic acids by HPLC or UHPLC.

Ludger has developed and validated technology to do this. The LudgerTag™ DMB kit (Cat No. LT-KDMB-A1) provides all that is required to release sialic acids from glycoproteins and label them with DMB. The kit contains reagents and materials for up to 22 glycoprotein samples (50-100 µg of glycoprotein per sample). Each labeling should work with approximately 10 pmol up to 2.5 nmol sialic acids per sample.

Included in the kit are a sialic acid reference panel (containing Neu5Ac, Neu5Gc, Neu5,7Ac2, Neu5,Gc9Ac, Neu5,9Ac2 and Neu5,7,(8),9Ac3Gc) and both NeuAc and NeuGc quantitative standards. These three standards can be labelled with DMB alongside the released sialic acid samples. Preparation of serial dilutions of the Neu5Ac and Neu5Gc quantitative standards enables quantitative analysis, by reference to standard curves. Information on how to do this is explained in the LudgerTag™ DMB kit guide. The Neu5Ac and Neu5Gc quantitative standards are also available to purchase separately if required as it can be useful to have additional amounts if you are setting up triplicate standard curves for your samples.

For positive controls, we recommend using fetuin glycoprotein which contains both Neu5Ac and Neu5Gc sialic acids. We also recommend a purified sialylated glycopeptide standard, the first in a range of Ludger BioQuant™ quantitative standards, as a positive control. This standard (Cat No. BQ-GPEP-A2G2S2-10U) is a complex biantennary N-linked glycan terminating in two N-acetylneuraminic acids. Using this standard will enable you to check the efficiency of glycan release, labeling and recovery and will give you confidence in the accuracy of your sialic acid measurements. Ludger also sells a standard which can be used for identification of Neu5,9Ac2 which is present in human erythropoietin (EPO). Formulation buffer can be used as a negative control.

Reference:

*Nguyen DH, Tangvoranuntakul P, and Varki A. (2005) Effects of Natural Human Antibodies against a Nonhuman Sialic Acid That Metabolically Incorporates into Activated and Malignant Immune Cells. J Immunol **175**:228-236.*

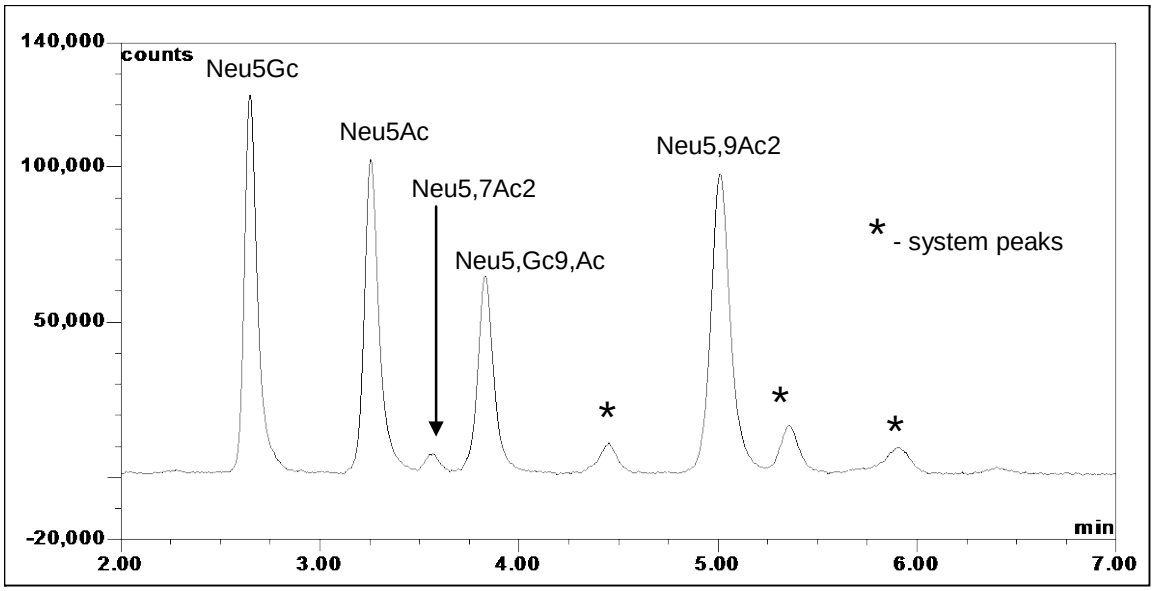

Figure 1: DMB-labeled sialic acid reference panel profiled on an LS-uR2 UHPLC column (Cat No. LS-UR2-2.1x100). The reference panel can be used as a system suitability standard prior to the sample run in order to check separation of glycans.

Ludger Products	Cat.No.
Release and label sialic acids with DMB:	
LudgerTag™ DMB Sialic Acid Labelling Kit	**LT-KDMB-A1**
Sialylated Positive Controls:	
Fetuin Glycoprotein (4 x 50 ug)	**GCP-FET-50U-X4**
Quantitative Glycopeptide Standard	**BQ-GPEP-A2G2S2-10U**
Neu5,9Ac2 standard	**CM-NEU5,9AC2-01**
N-acetylneuraminic acid (Neu5Ac or NANA) quantitative standard	**CM-NEU-AC-01**
N-glycolylneuraminic acid (Neu5Gc or NGNA) quantitative standard	**CM-NEU-GC-01**
HPLC analysis:	
LudgerSep™ R1 HPLC Column	**LS-R1-4.6x150**
UHPLC analysis:	
LudgerSep™ uR2 UHPLC Column	**LS-UR2-2.1x100**