Ludger Monosaccharide Analysis Guide

Monosaccharide analysis is a regulatory requirement laid out in the ICH Q6B guidelines for characterisation of biopharmaceuticals. This information can be used at all stages of drug development as a method of determining the type of glycosylation (N-linked and/or O-linked) and the extent to which glycosylation has occurred. It can also be used to demonstrate consistency between batches for QC lot release during the manufacturing process.

A widely used method for monosaccharide analysis is as follows:

- Release of monosaccharides from the glycoprotein by mild acid hydrolysis.
- Fluorescent labelling of released monosaccharides with 2-aminobenzoic acid (2AA).
- Relative quantitative analysis of 2AA-labelled monosaccharides by HPLC column or UHPLC.

The LudgerTag™ Monosaccharide Release and Labelling kit (Cat No. LT-MONO-96) provides all that is required to release neutral and amino monosaccharides from glycoproteins and label with 2AA. The kit contains reagents and materials for up to 96 glycoprotein samples (typically, around 50 µg of glycoprotein per sample). It includes a quantitative standard (monomix) containing 6 monosaccharides. The monomix standard enables instrument calibration in order to quantitatively determine the monosaccharide components in your glycoprotein. We recommend a five point calibration curve to be used for the monomix standard. Information on how to do this is explained in the kit guide.

Xylose, a monosaccharide typically found in plants and insects but not mammalian or yeast expression vectors, is also included. It can be used as an internal standard (i.e. added to each sample before labelling). This allows compensation for any pipetting/sample preparation errors that may have occurred during sample processing. We also recommend a purified glycopeptide standard, the first in a range of Ludger BioQuant™ quantitative standards, as a positive control. This quantitative 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 monosaccharide measurements.

We offer two choices of column for analysis of the labelled monosaccharides dependent on whether you are using HPLC or UHPLC systems in your laboratory. If using an HPLC, the LudgerSep™ R2 column (Cat No. LS-R2-4.6x150) gives very good separation of the seven main monosaccharides found in most N-link and O-link glycans. If you have an UHPLC system we recommend using the LudgerSep™ uR2 column (Cat No. LS-UR2-2.1x50) which can perform an 8 minute separation per sample. See Figure 1.
Figure 1: 2AA-labeled monosaccharide standards profiled on a LudgerSep uR2 UHPLC column (Cat No. LS-UR2-2.1x50). Peaks for the following monosaccharides appear within 8 minutes; glucosamine (GlcN), galactosamine (GalN), galactose (Gal), mannose (Man), glucose (Glc), xylose (Xyl) and fucose (Fuc).

**Ludger Products (can be ordered via e-mail at info@ludger.com)**

**Release monosaccharides and label with 2AA:**
LudgerTag™ Monosaccharide Release and Labeling Kit
Cat No. LT-MONO-96

**Positive control:**
Quantitative glycopeptide standard
Cat No. BQ-GPEP-A2G2S2-10U

**For HPLC analysis:**
LudgerSep™ R2 HPLC Column
Cat No. LS-R2-4.6x150

**For UHPLC analysis:**
LudgerSep™ uR2 UHPLC Column
Cat No. LS-UR2-2.1x50

**Solvent:**
LudgerSep™ R BPT solvent (x10 concentrate)
Cat No. LS-R-BPTX10