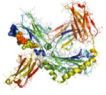
Workflow for Level 2 Exoglycosidase digestion followed by HILIC-UPLC

Ludger
www.ludger.com



Glycoprotein or GSL

Include process controls for monitoring glycan release and labelling

- Well characterised glycoprotein standard – Ludger human IgG positive control [GCP-IGG-100U]
- Formulation buffer – negative control

Enzymatic or Chemical Release of Glycans

Include system suitability, reference and calibration standards

- Fluorescently labelled Glucose Homopolymer (GHP) for Glucose Units (GU) allocation
- Fluorescently labelled glycan standards common to analysed samples

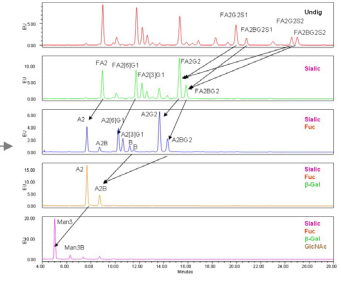
Fluorescent labelling and clean-up

Exoglycosidase sequencing and clean-up

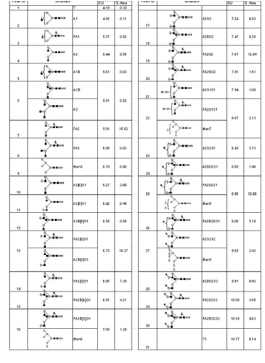
Example workflow for N-glycans can include:

- Sialidasespecific for α2-3, 6, 8, 9 sialic acids
- Beta galactosidase specific for β1-4 galactose
- Fucosidase specific for α1-3and4 fucose
- Fucosidase specific for α1-6>2 fucose
- N-acetylglucosaminidase specific for beta GlcNAc
- Alpha galactosidase specific for αGal
- Alpha mannosidase specific for α1-2,3,6 Mannose

HILIC UHPLC



HILIC-UHPLC-FLR profiles for test sample before and after exoglycosidase digestion



N-glycan structures identified in the test sample

Level 1 HILIC profiling

Level 2 Exoglycosidase digestion followed by HILIC-UPLC