Sample 1: Pharmacology

N-Glycan Analysis of Biotherapeutic

Glycoproteins Using AdvanceBio

Gly-X 2-AB Express Sample

Preparation and LC/FLD/MS

N-glycan analysis simplified and standardized

The location and structure of N-linked glycans plays a critical role in the

pharmacology of therapeutic proteins, potentially affecting immunogenicity,

pharmacokinetics, and pharmacodynamics.

2-AB (2-aminobenzamide) is a well-established tag that has been used to generate

N-glycan data for more than 20 years. Agilent AdvanceBio Gly-X 2-AB Express

is a high-performance N-glycan sample preparation platform1 with a simplified

workflow, using a five-minute in solution deglycosylation step followed by 2-AB

labeling on a solid-state matrix. Excess dye is washed away with acetonitrile before

eluting labeled samples with DI water without requiring sample drying. Samples are

ready for UHPLC/FLD/MS in 2 hours or less using the AdvanceBio Glycan Mapping

column for hydrophilic interaction liquid chromatography (HILIC) followed by relative

quantitation. In addition, a wide range of 2-AB-labeled N-glycan standards are

available to calibrate N-glycan separations and help identify N-glycan species.

Getting started with Gly-X 2-AB Express:

Tips for optimal results

Glycoprotein sample preparation considerations

Glycoprotein samples should be prepared at a maximum

concentration of 2 mg/mL in a low salt neutral buffer free of

detergents. Higher concentration samples should be diluted

in water or 50 mM HEPES, pH 7.9.

– Maximum concentration: 2 mg/mL.

– Maximum amount of protein per reaction: 40 μg

(for example, 20 μL of each 2 mg/mL solution).

Higher quantities of protein could be used for mAbs

(up to 100 μg) but data linearity should be assessed

when loading more than 40 μg.

– Buffer: Low salt (~150 mM) neutral buffer without

detergents. Sample can be diluted with water or

50 mM HEPES, pH 7.9.

– A 10 kDa molecular weight cut-off spin centrifugal filter

is recommended when the sample salt concentration is

higher than 150 mM.

Incubation and cleanup hardware

During the Gly-X 2-AB Express sample preparation workflow,

samples are heated to 90 °C for protein denaturation, 50 °C

for PNGase F digestion, and 65 °C for 2-AB labeling. We

recommend using a thermocycler or dry block heater when

heating the samples in the 96-well plate provided using the

suggestions below.

The workflow employs a simple, vacuum-driven cleanup.

If you wish to use an equivalent heater, vacuum manifold

or pump other than the models suggested in this table,

validation may be needed.

Sample 2: Radiooncology

In the ￼Anatomy Review￼ work area, you can review and correct the auto-generated organ structures of the session patient model (session image) and compare them with the organ structures of the intent patient model (reference image).

In the structure list, the structures are divided into influencer structures (that is, contours of organs that have a strong influence on target structures in terms of position, shape, and dosimetric impact) and other organ structures.

Special emphasis is placed on reviewing the so-called influencer structures.

They are organs that have a strong influence on target structures in terms of position, shape, and dosimetric impact.

The purpose of influencer structures is to guide the creation of the target structures and the creation of the adaptive plan.

The purpose of reviewing the influencer structures is to ensure that they are contoured correctly and according to the same guidelines that were applied while contouring the planning images.

Based on structure propagation from the RT intent (reference image with intent patient model) or AI segmentation algorithms, the work area shows the following: Session Image, Reference Image, Session patient model, Intent patient model, All organ structures (influencer and other) of the RT intent, Verification structures (body, high-density structures).