PROzyme®

# CERTIFICATE OF ANALYSIS

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|---|---|
| PRODUCT NAME: | GLYKO® 2-AA-(HUMAN IgG N-LINKED GLYCAN LIBRARY) |
| PRODUCT CODE: | GKSA-005 |
| LOT NUMBER: | DP14A2701 |
| PACK SIZE: | 200 pmol (qualitative standard for glycan identification) |
| FORM: | Dry solid |
| STORAGE: | Store in the dark at -20°C before and after reconstitution |
| EXPIRATION: | March 2024 (extended from prior exp. date based on re-assay), may be used for 1 year after reconstitution |
| RE-ASSAY DATE: | March 2019 |
| STRUCTURE: | The 2-AA-(Human IgG N-Linked Glycan Library) consists of complex biantennary oligosaccharides consistent with N-glycans on normal human IgGs[1,2,3] with the reducing termini derivatized with the fluorescent dye, 2-AA (2-aminobenzoic acid). |

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## Quality Control:

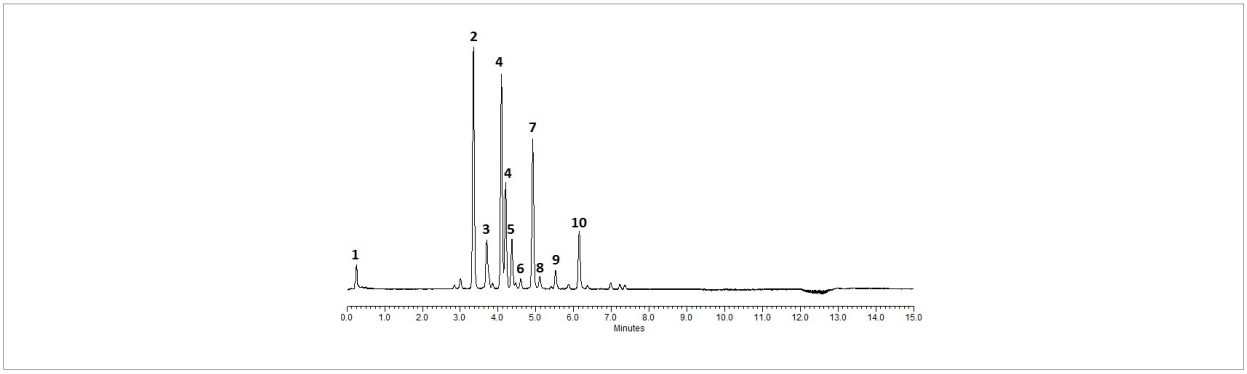



**Figure 1 - UPLC® Results**: 8 pmol (1 µl, aqueous) of the 2-AA-labeled[4] library was injected on a Waters ACQUITY UPLC® H Class System utilizing a 15-minute method under the conditions below (see Table 1 for peak identification):

| Time (min) | Flow (ml/min) | % ACN | % Buffer |
|---|---|---|---|
| 0.0 | 1.0 | 75.0 | 25.0 |
| 1.0 | 1.0 | 75.0 | 25.0 |
| 12.0 | 1.0 | 52.5 | 47.5 |
| 12.1 | 0.5 | 40.0 | 60.0 |
| 12.5 | 0.5 | 40.0 | 60.0 |
| 12.6 | 0.5 | 75.0 | 25.0 |
| 12.7 | 1.0 | 75.0 | 25.0 |
| 15.0 | 1.0 | 75.0 | 25.0 |

Column: Waters ACQUITY UPLC BEH Glycan Column (1.7 µm, 2.1 x 100 mm)
ACN: Acetonitrile
Buffer: 100 m*M* ammonium formate, pH 4.4
Flow rate: As stated in table, in ml/min
Temperature: 60°C
Max Pressure: 15,000 psi
Fluorescence Detection: $\lambda_{ex}$ = 352 nm, $\lambda_{em}$ = 435 nm

## Table 1: Peak Identification of 2-AA-Labeled Human IgG N-Linked Glycan Library.

| Peak Number | Glycan Identification |
|---|---|
| 1 | Free 2-AA (reagent peak) |
| 2 | Asialo-, agalacto- biantennary, core-substituted with fucose [NGA2F/G0F] |
| 3 | Asialo-, agalacto- biantennary, core-substituted with fucose and with bisecting N-Acetylglucosamine [NGA2FB/G0FB] |
| 4 | Asialo, mono-galactosylated biantennary, core substituted with fucose [NA2G1F/G1F] |
| 5 | Asialo, mono-galactosylated biantennary, core substituted with fucose and with bisecting N-Acetylglucosamine [NA2G1FB /G1FB] |
| 6 | Asialo-, galactosylated biantennary [NA2/G2] |
| 7 | Asialo-, galactosylated biantennary, core-substituted with fucose [NA2F/G2F] |
| 8 | Asialo-, galactosylated biantennary, core-substituted with fucose and with bisecting N-Acetylglucosamine [NA2FB/G2FB] |

| Peak Number | Glycan Identification |
|---|---|
| 9 | Mono-sialylated, mono-galactosylated biantennary, core substituted with fucose [G1SF1] |
| 10 | Mono-sialylated, galactosylated biantennary, core substituted with fucose [A1F] |

**Structural Analysis:** The purity and structural integrity of the glycan library was assessed by UPLC analysis. The identity of the unlabeled glycan library is confirmed by MALDI-TOF mass spectrometry [5,6], ESI-MS or LC-MS. Agreement was found between the results from mass spectrometry and UPLC[7].

**Application:**

- Qualitative standard for various analytical procedures
- As a migration standard for liquid chromatography

**Handling & Reconstitution:** The labeled library is shipped as a dried solid. Use ultra-pure water or an aqueous buffer to dissolve the glycan (see Directions for Use for suggested volume).

Allow the unopened vial to reach ambient temperature and tap on a solid surface to ensure that most of the material is at the bottom of the vial. Gently remove the cap, add the desired volume of water or buffer, re-cap and mix thoroughly to redissolve all the oligosaccharide. For maximal recovery, ensure that the cap lining is also rinsed and centrifuge the reconstituted vial briefly before use.

Make sure that any glassware, plasticware, solvents or reagents used are free of glycosidases and carbohydrate contaminants.

Minimize exposure to elevated temperatures or extremes of pH.
Store the reconstituted glycan at -20°C.
Allow the vial to equilibrate to ambient temperature before use.

**Directions For Use:** The amount of 2-AA-labeled glycan library injected on a UPLC column is typically 6-9 pmol of total glycan. For our Quality Control testing, one vial was dissolved in 30 µl of water and 1 µl injected on the ACQUITY column. For larger injection volumes or other LC systems we recommend further dilution as necessary for compatibility with the mobile phase. For suggested methods see Rapid UPLC Methods for Screening Labeled N-Glycans (TNGP101) available at:

*www.prozyme.com/tech_notes.html*

## REFERENCES


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