KORNBERG SEQNOSTICS

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2-AB BIANTENNARY & HIGH MANNOSE PARTITIONED LIBRARY

Product Code:
KNBS-9520

Pack Size:
200 pmol (qualitative standard for glycan identification) and 1 each 2-AB-(Fucosyl Biantennary Library), 1 each 2-AB-(Afucosyl Biantennary Library), 1 each 2-AB-(High Mannose Library)

Form:
Glycan Library - dry solid, Human IgG Glycoprotein - dry solid

Purity:
The purity and structural integrity of the glycan library was assessed by UPLC4 (GU values) and MALDI-TOF mass spectrometry5,6 or LC-MS. Good agreement was found between the results from mass spectrometry and UPLC.

Storage:
Store at -20°C in the before and after reconstitution.

Reconstitution:
Use HPLC-grade water or an aqueous buffer to dissolve the glycan library. Store the reconstituted library at -20°C in working aliquots; avoid multiple freeze/thaw cycles. To be used within 1 year after reconstitution

Introduction:
The 2-AB-(Biantennary & High Mannose Partitioned Library) consists of 3 blended libraries of N-linked glycans whose reducing termini are derivatized with the fluorescent dye, 2-AB (2-aminobenzamide). The libraries were partitioned to minimize overlap of peaks to facilitate glycan peak identification.

Application:
Suitable for various analyticl procedures. Used as a migration standard for liquid chromatography.

Procedure:
1. Bring the unopened vial to Room Temperature. Tap on a solid surface to ensure that most of the material is collected at the bottom of the vial.
2. Gently remove the cap, add the desired volume of water or buffer, re-cap and mix thoroughly to re-dissolve all the oligosaccharide.
3. Centrifuge the reconstituted vial briefly before use.
4. For maximal recovery, ensure that the cap lining is also rinsed. Centrifuge the reconstituted vial briefly before use.
5. The amount of 2-AB-labeled library standard injected on a UPLC column is typically 6 – 9 pmol of total glycan. We recommend further dilution as necessary for compatibility with the mobile phase.

UPLC Running Conditions:
6 - 9 pmol (1 µl) of each 2-AB labeled glycan library was injected on a Waters ACQUITY UPLC® H Class System utilizing a 15-minute method under the conditions below:

Time (min)	%ACN	%Buffer	Flow Rate
0	75	25	1
12	52.5	47.5	1
12.1	40	60	0.5
12.5	40	60	0.5
12.6	75	25	0.5
12.7	75	25	1
15	75	25	1

ACN: acetonitrile Buffer: 100 mM ammonium formate pH 4.4
Flow rate: above, in ml/min Temperature: 60ºC Max Pressure: 15,000 psi
Fluorescence Detection: 8ex = 330 nm , 8em = 420 nm

Typical Reference Graph

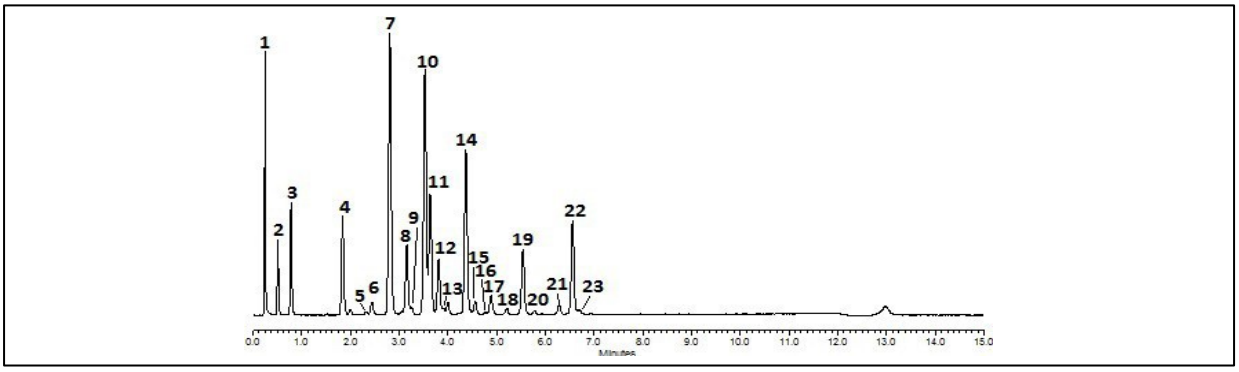


Figure 1 - UPLC® of AB Fucosyl Biantennary Library: Peaks confirmed by mass spectrometry listed in Table 1.

Table 1 - Peak Identification of 2AB Fucosyl Biantennary Library.

Peak No	Glycan Identification
1	Free Dye (2-AB)
2	6-α-fucosyl chitobiose
3	4'-β-mannosyl chitobiose with core fucose
4	Conserved trimannosyl core, substituted with fucose
5	Asialo-, agalacto-, biantennary complex N- Glycan with core fucose, -1 N-Acetylglucosamine
6	Asialo-, agalacto- biantennary
7	Asialo-, agalacto- biantennary with core fucose
8	Asialo-, agalacto- biantennary with core fucose and with bisecting N-Acetylglucosamine
9	Asialo-, mono-galacto- biantennary
10 + 11	Asialo-, mono-galacto- biantennary with core fucose
12 + 13	Asialo-, mono- galactosylated biantennary with core fucose and bisecting N- Acetylglucosamine
14	Asialo-, galactosylated biantennary with core fucose
15	Asialo-, galactosylated biantennary with core fucose and bisecting N- Acetylglucosamine
16 + 17	Mono-α(2-6)-sialylated, mono-galactosylated, biantennary with core fucose
18	Mono-α(2-6)-sialylated, galactosylated biantennary
19	Mono-α(2-6)-sialylated, galactosylated biantennary, with core fucose
20	Mono-α(2-6)-sialylated, galactosylated biantennary, with core fucose and bisecting N- Acetylglucosamine
21	Di-α(2-6)-sialylated, galactosylated biantennary
22	Di-α(2-6)-sialylated, galactosylated biantennary with core fucose
23	Di-α(2-6)-sialylated, galactosylated biantennary with core fucose and bisecting N- Acetylglucosamine

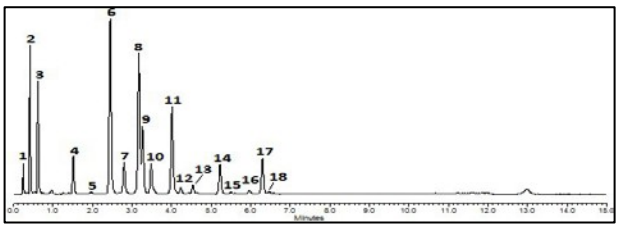


Figure 2 - UPLC® of 2-AB Afucosyl Biantennary Library: Peaks confirmed by mass spectrometry listed in Table 2.

Table 2 - Peak Identification of 2AB Afucosyl Biantennary Library.

Peak No	Glycan Identification
1	Free Dye (2-AB)
2	Chitobiose
3	4'-β-mannosyl chitobiose
4	Conserved trimannosyl core
5	Asialo-, agalacto-, biantennary complex N- Glycan, -1 N-Acetylglucosamine
6	Asialo-, agalacto- biantennary
7	Asialo-, agalacto- biantennary with bisecting N-Acetylglusosamine
8 + 9	Asialo-, mono- galactosylated biantennary
10	Asialo-, mono- galactosylated biantennary with bisecting N- Acetylglusosamine
11	Asialo-, galactosylated biantennary
12	Asialo-, galactosylated biantennary with bisecting N-Acetylglucosamine
13	Mono-α(2-6)-sialylated, mono-galactosylated, biantennary
14	Mono-α(2-6)-sialylated, galactosylated biantennary
15	Mono-α(2-6)-sialylated, galactosylated biantennary with bisecting N- Acetylglucosamine
16 + 17	Di-α(2-6)-sialylated, galactosylated biantennary
18	Di-α(2-6)-sialylated, galactosylated biantennary with bisecting N- Acetylglucosamine

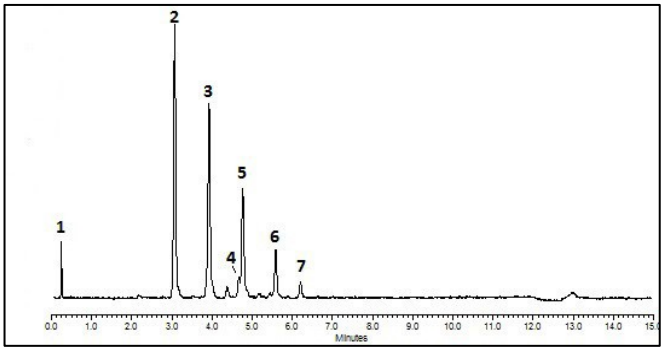
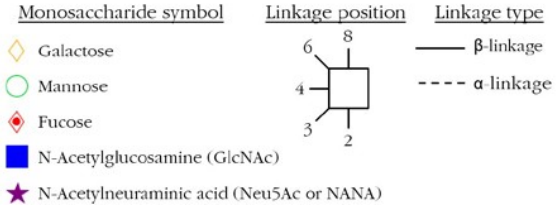


Figure 3 - UPLC® of 2-AB High Mannose Library: Peaks confirmed by mass spectrometry listed in Table 3.

Table 3 - Peak Identification of 2AB High Mannose Library.

Peak No	Glycan Identification
1	Free Dye (2-AB)
2	Oligomannose 5
3	Oligomannose 6
4 + 5	Oligomannose 7
6	Oligomannose 8
7	Oligomannose 9

Structure Key:



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Precautions:
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; high temperatures or low pH will cause desialylation. High pH will cause epimerization of the reducing terminal GlcNAc.