

2-AB Labeling of Glycans

MATERIALS

- Glycoclean S-Cartridges (Prozyme, GKI-4726)
- 2-Aminobenzamide (Sigma, A89804)
- Sodium Cyanoborohydride (Sigma, 156159)
- Dimethyl Sulphoxide (Sigma, 276855)
- Glacial Acetic Acid
- Acetonitrile (Fisher, A998-4)
- 96% Acetonitrile
- 30% Acetic Acid
- Sodium Acetate Trihydrate (NaOAc) (VWR, JT3462-1)

Preparation of 1L of 200 mM NaOH:

- 10.4 mL of 50% NaOH

Dissolve the 50% NaOH and bring the volume to 1 L. Thoroughly mix the solution and sparge the solution with Helium for 15 min to remove dissolved gases.

Preparation of 1L of 200 mM NaOH with 500 mM NaOAc:

- 68 g of Sodium Acetate Trihydrate
- 10.4 mL of 50% NaOH

Dissolve the Sodium Acetate Trihydrate in Milli-Q water that has been filtered through a 0.2 μm filter. Then add the 50% NaOH and bring the volume to 1 L. Thoroughly mix the solution and sparge the solution with Helium for 15min to remove dissolved gases.

PROCEDURE:

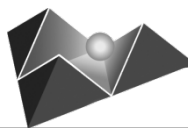
1. Prepare fresh 2AB reagent as follows (See table below on how to prepare):

- a. Measure out the 2AB in a clean microfuge tube.
- b. In a separate tube measure out the Sodium Cyanoborohydride.
- c. In a third tube combine 130 μL of DMSO and 70 μL of Glacial Acetic Acid (65:35), mix by vortexing and then transfer 100 μL of the mixture to the microfuge tube containing the 2AB.
- d. Dissolve the 2AB with the DMSO:Acetic Acid by vortexing and then transfer the entire contents to the tube containing the Sodium Cyanoborohydride and mix. Sonicate, if necessary, until it is fully dissolve.



2-AB Preparation	Reagent	Amount
2-Aminobenzamide (2-AB)		6-6.5 mg
Sodium Cyanoborohydride		6.5-6.8 mg
DMSO		130 μ L
Glacial Acetic Acid		70 μ L

2. Transfer an aliquot of sample into a 500 μ L microfuge tube and dry by lyophilization. It is important that the samples are free of water.
3. Add 10 μ L of the 2AB reagent to the sample tube, mix, and then incubate on a heating block at 65 °C for 2.5 hrs.
4. After labeling is complete remove excess 2AB reagent using a Glycoclean S-cartridge as outlined below:
 - a. Glycoclean S-cartridge Cleaning:
 - i. Wash with 3-4 mL of water.
 - ii. Wash with 12-15 mL of 30% Acetic Acid
 - iii. Wash with 15-20 mL of 100% Acetonitrile (AVOID water at this point as it will elute the labeled glycans)
 - b. Removing Excess 2AB reagent:
 - i. Make sure the membrane of the cartridge is wet with 100% Acetonitrile and then add the entire sample to the center of the S-cartridge membrane and let it absorb for 10 min.
 - ii. Rinse the derivatization tube with 100 μ L of 100% ACN and add it to the cartridge and leave it to absorb for 10 minutes.
 - iii. Wash S-cartridge with 2 mL of 100% Acetonitrile
 - iv. Wash the S-cartridge with 12-15ml 96% Acetonitrile (Removes Excess 2AB Reagent)
 - v. Remove excess acetonitrile from the bottom of the cartridge, and then place the cartridge over a clean 2 mL microfuge tube.
 - vi. Elute the labeled glycans by passing 500 μ L of water over the S-cartridge membrane and collecting it in a 2 mL centrifuge tube. Wash another TWO times with 500 μ L of water and collect it in the same tube.
 - vii. Lyophilize the collected washes to dryness under vacuum.
5. Bring the dried sample up in a known volume of water, and then take an aliquot for HPLC analysis. Alternatively samples can be stored at -20 °C away from light.



- a. Inject 1 μg for a known protein such as RNase A, Fetuin, or IgG. For an unknown protein inject an amount based on the results from the monosaccharide analysis on the original sample.

HPLC OF 2-AB LABELLED GLYCANS:

- Colum:
 - Dionex CarboPac PA1 column 4 mm x 250 mm, 4 μm , with 4 mm x 50 mm Guard
- Solvents:
 - A: Milli-Q Water
 - B: 200 mM NaOH
 - C: 200 mM NaOH with 500 mM NaOAc
 - Initial conditions of 50% A and 50% B at 1.0 mL/min
- Fluorescence Detector:
 - Excitation: 330 nm
 - Emission: 420 nm
 - Gain: 0.5
 - Sensitivity: High
- Gradient Settings:

Time (min)	Water	200 mM NaOH	200 mM NaOH 500 mM NaOAc
0	50%	50%	0%
5	50%	50%	0%
25	50%	40%	10%
75	50%	0%	50%
80	50%	0%	50%
80.5	50%	50%	0%
95	50%	50%	0%