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.

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

2. Transfer an aliquot of sample into a 500 µL microfuge tube and dry by lyophilization. It is important that the samples are free of water.

3. Add 10 µL of the 2AB reagent to the sample tube, mix, and then incubate on a heating block at 65 ^{o}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 absorbe for 10 min.
      ii. Rinse the derivatization tube with 100 µL 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 µL of water over the S-cartridge membrane and collecting it in a 2 mL centrifuge tube. Wash another TWO times with 500 µL 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 ^{o}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, 4µm, 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%

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