Release of N-Glycans Using PNGase F

MATERIALS

- PNGase F (Glycerol Free) (NEB, 500,000 units/mL, P0705S or P0705L)
- 10X Denaturing Buffer
- 10X NP-40
- 10X G7 Buffer
- Sep Pak C18 Column (100mg, CAT# 023590 from Waters)
- PGC Column (charcoal, 25mg, Hypersep hypercarb, CAT# 60106-304 from Thermo Scientific)
- 5% Acetonitrile with 0.1% TFA
- 15% Acetonitrile with 0.1% TFA
- 30% Acetonitrile with 0.1% TFA

10X Denaturing Buffer:
5% SDS and 400 mM DTT

- 0.250 g of SDS
- 3.08 g of DTT

Dissolve in 5 mL of water, separate into 500 µL aliquots and store at -20 ^{o}C.

10X NP-40:
10% NP-40

- 10 mL of NP-40

Dissolve in 100 mL Milli-Q water, store at 8 ^{o}C

10X PNGase F Buffer:
500 mM Sodium Phosphate pH 7.5 with 0.02% Sodium Azide

- 1M Na_2HPO_4 with 0.04% Sodium Azide
- 1M NaH_2PO_4 with 0.04% Sodium Azide

Take 20.25 mL of 1 M Na_2HPO_4 and add 4.75 mL NaH_2PO_4 adjust pH to 7.5 and dilute 50 mL with Milli-Q water. Check pH again and adjust to 7.5 with either Na_2HPO_4 or NaH_2PO_4.

PROCEDURE:

NOTE: Do not Use more than 10 million cells for N-glycan release. If starting from protein it is suggested that a monosaccharide analysis be performed to determine the amount of glycans present.

1. Dissolve a known amount of sample in water.

NOTE: If starting from cells first rupture the cells by sonication for 1 min with 30 s pulses. Perform protein estimation as well as a monosaccharide analysis. If starting from protein, re-suspend in water and perform both protein estimation and monosaccharide analysis.

2. Next add enough 10X Denaturing Buffer to the sample to make a solution with a final concentration of 1X.

3. Denature the proteins by heating the sample at 100 ^{o}C for 10 min.

4. Remove the sample from heat, allow it to cool to room temperature and then centrifuge briefly.

5. Add enough 10X NP-40 to the sample to make a solution with a final concentration of 1X. Leave the sample to incubate for 30-45 min on the benchtop. Mix the sample every 10-15 min.

6. Now add enough 10X PNGase F Buffer to the sample to make a solution with a final concentration of 1X.

7. Add 500-1,500 NEB units (1-3 µL) of PNGase F stock to the sample. Gently mix the solution and then incubate the sample at 37 ^{o}C overnight (16-18 hr) with continuous tumbling.

8. Purification of the N-glycans from the mixture is performed by passing the reaction mixture first over a SepPak C18 column and then a PGC column. Arrange a SepPak C18 cartrige so that the elutent enters the PGC cartridge.

NOTE: It is important to precondition the SepPak and PGC columns prior to using. See below for conditioning instructions.

NOTE: The flow of solution through the column should only be under gravity during the entire load and wash process.

9. Load the reaction mixture onto the SepPak C18 column. Rinse the reaction tube with 1 mL of Milli-Q water and load onto the column. Wash the column with a further 2 mL of Milli-Q water in 1 mL increments.

10. Allow all of the eluent from the SepPak C18 column pass through the PGC column and then wash the PGC column with 3 mL of 5% Acetonitrile with 0.1% TFA.

11. Elute the N-glycans by washing the column first with 2 mL of 15% Acetonitrile with 0.1% TFA and then with 1 mL of 30% Acetonitrile with 0.1% TFA. Pool both the 15% and 30% fractions and lyophilize the samples to dryness.

website: glycotech.ucsd.edu | e-mail: glycotech@ucsd.edu | phone: (858) 822-1039

12. Perform a monosaccharide analysis on a portion of the sample to confirm the presence of N-Glycans.

COLUMN PREPARATION:

Sep Pak C-18:

- wash with 3 mL of 10% acetic acid
- wash with 3 mL of 50% Methanol
- wash with 3 mL of 100% Methanol
- wash with 3 mL of 72% Isopropanol + 28% Methanol + 0.1% formic acid
- wash with 2 mL of anhydrous ethyl acetate
- wash with 3 mL of Chloroform
- wash with 3 mL of 100% Methanol
- wash with 3 mL of 50% Methanol
- wash with 3 mL of water

PGC:

- wash with 3 mL of acidified water (0.1% TFA in water)
- wash with 3 mL of 50% acetonitrile
- wash with 3 mL of 100% acetonitrile
- wash with 3 mL of 50% acetonitrile
- wash with 3 mL of acidified water (0.1% TFA in water)
- wash with 3 mL of Milli-Q water

For all the washes described above pressure can be applied to the column (syringe attached to Teflon tubing). Special care, however, should be taken not to introduce any air gaps in the cartridges, which can reduce the performance of the cartridges.

website: glycotech.ucsd.edu | e-mail: glycotech@ucsd.edu | phone: (858) 822-1039