Preparing Cells and Tissues for N- and O-glycan Release

MATERIALS

- Denaturing Buffer
- 10% Ethanol
- 50% Methanol
- Milli-Q water
- 10% Dithiothreitol
- 1:2 Chloroform:Methanol
- 1:1 Chloroform:Methanol
- 2:1 Chloroform:Methanol
- High speed homogenizer
- Sodium Dodecylsulphate (Fisher, BP166-500)

Denaturing Buffer:

20 mM HEPES pH 7.5 with 1% SDS

- 0.2383 g of HEPES
- 0.5 g of SDS

Dissolve the HEPES and SDS in 30 mL of Milli-Q water with stirring. Titrate the solution to pH 7.5 with sodium hydroxide and bring the final volume to 50 mL with Milli-Q water. Check the pH and readjust if necessary.

PROCEDURE:

NOTE: For glycans analysis of mammalian tissues or cells, 100-400 mg of tissue or 50-100 x 10^6 cells is sufficient for a series of MS analyses. These include sequential exoglycosidase digestions, MS/MS studies and linkage analyses.

1. For 100 mg of tissue add 2-4 mL of Denaturing Buffer and homogenize the sample to breakdown the tissue to a fine particle size. For 50-100 x10^6 cells add 1 mL of denaturing buffer and sonicate. Aliquots of 500 µL of buffer can be added as required to lower the viscosity of the solution.

NOTE: To clean the homogenizer for tissues wash sequentially with 25-30 mL of 10% Ethanol, 50% Methanol and Milli-Q wate before and after homogenization. For Cells you can use a sonicator for 3-5 min with 30 min pulses. Avoid excessive frothing of the sample.

2. Place the homogenized material in a boiling water bath for 10 min.

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3. Allow the sample to cool to room temperature and add 10% DTT to obtain a final concentration of 0.5% DTT. Keep the solution at room temperature for 1 hr with occasional vortexing. Avoid excessive frothing of the sample.

4. Freeze the sample on dry ice and lyophilize the extract. The samples should be completely dry before proceeding to the next step.

Lipid and SDS Extraction:

1. Extract the lipids and detergent by sequential extraction using 2:1, 1:1, and 1:2 Chloroform:Methanol mixtures. Extractionusing a particular solvent sixture should be done three times using half the volume of the sample. Thoroughly vortex the samples to break down any lumps. If the sample becomes too viscous add 500 µL aliquots of solvent to the sample.

2. Sonicate the sample briefly if there is a problem suspending the materials in organic solvents.

3. Centrifuge the sample at 4000 rpm for 5 min at 10 ^{o}C. Remove the organic layer and re-extract the sample.

4. Pool the organic layers and kept for possible glycolipid analysis.

5. Finally the lipid extracted pellets should be dried down completely using a low flow of dry nitrogen. Take special care when drying down samples as excessive drying can cause the sample to fly away under the nitrogen flow.

6. Resuspend the dried samples in the original volume of water and sonicate to create a uniform suspension.

7. Perform a protein estimation of the sample and a total monosaccharide analysis.

8. Use the results of the monosaccharide analysis as a guideline for the amount of material to be taken for N- and O-glycan analysis.

9. Combination of the results also provide ideas for further purification of samples, such as the removal of glycogen which is often found is several cell and tissue samples.

10. Uronic acids are also observed in cells or tissue extract and this material can also be used for GAG extraction analysis.

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