



MALDI-TOF Analysis of Glycans

MATERIALS

- N- or O-glycan solution (1 $\mu\text{g}/\mu\text{L}$)
- Super-2,5-Di hydroxyl benzoic acid or gentisic acid (SDHB)
- Methanol
- Dihydroxy benzoic acid (DHB) (Sigma, 149357)
- 5-O-methoxyl salicylic acid (Sigma, 288306)

Preparation of Super-2,5-Dihydroxyl benzoic acid:

- 77 mg DHB
- 15 mg 5-O-methoxy salicylic acid

Add both the DHB and 5-O-methoxy salicylic acid to a glass vial and add 1 mL of Methanol and mix to dissolve. Wrap the vial in aluminum foil and store at $-20\text{ }^{\circ}\text{C}$.

PROCEDURE:

1. For analysis of underivatized glycans mix the sample in a 1:1 (v/v) ratio with SDHB and spot 1 μL on a stainless steel MALDI plate.
2. Mass spectra can be acquired in both positive and /or negative mode.
3. For better results on highly sialylated glycans, methylation is essential as permethylated glycans give strong signals and protects against desialylation on the mass spec.
4. For permethylated glycans, dissolve the sample in 200 μL methanol. Remove and dry down 50 μL in a microcentrifuge tube. Re-dissolve the sample in 5 μL of methanol and add 1 μL of water containing 1% TFA, mix thoroughly. Mix 1 μL of this sample with 1 μL of SDHB, spot on MALDI plate and allow spot to dry.
5. Mass spectra for permethylated glycans should only be acquired in the positive mode.