

## Permethylation for Glycans

Ver. 20170821

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>10 pmol of glycan

- ↓ Lyophilize in 1 mL Pierce vial
- ↓ 75  $\mu$ L of DMSO/NaOH
  - 2 spatulafuls granular NaOH (Wako, for organic synthesis)
  - ↓ Grind by pestle in mortar
  - ↓ Add 800  $\mu$ L of 99% DMSO-1% DDW (v/v) (1 mL separate storage)
  - ↓ Mix and use immediately
- ↓ Move to draft
- ↓ 50  $\mu$ L of CH<sub>3</sub>I measured by marked Pasteur pipet (marked at 50  $\mu$ L position)
- ↓ Close the lid tightly
- ↓ Shake on shaker, r.t., 30 min

(As the reaction proceeds, the solution turns cloudy)

- ↓ Spin down briefly
- ↓ 300  $\mu$ L ice-cold DDW
  - (low temp. prevent methyl ester dissociation on sialic acid)
  - (use 250  $\mu$ L ice-cold 20% AcOH in the case of SepPak C18 purification)
- ↓ Mix by Vortex

(SepPak C18 purification is suitable for sulfated or phosphorylated glycans.)

- ↓ 400  $\mu$ L CHCl<sub>3</sub>
- ↓ Mix well by hand shaking
- ↓ Remove the water layer
- ↓ 500  $\mu$ L DDW
- ↓ Mix well by hand shaking
- ↓ Remove the water layer
- ↓ Repeat the water extraction two more times
- ↓ Concentrate by concentrator
- ↓ Dry up by freeze dryer
- ↓ Dissolved in 50  $\mu$ L 50% MeOH
- ↓ Transfer into LC-MS vial
- ↓ Co-wash by 50  $\mu$ L 50% MeOH
- ↓ Transfer into LC-MS vial

100  $\mu$ L permethylated sample in 50% MeOH

- ↓ Storage at -20°C

SepPak C18 purification (For sulfated or phosphorylated glycans)

The reaction mixture

- ↓ Neutralize with 250  $\mu$ L ice-cold 20% AcOH
  - ↓ Load to pretreated SepPak C18 cartridge
    - pretreatment
    - ↓ Wash with 1 mL CH<sub>3</sub>CN
    - ↓ Wash with 1 mL DDW
  - ↓ Wash with 3 mL DDW
  - ↓ Elute with 1 mL 80% CH<sub>3</sub>CN-0.1% AcOH-20%DDW
  - ↓ Concentrate by concentrator
  - ↓ Lyophilize
  - ↓ Dissolved in 50  $\mu$ L 50% MeOH
  - ↓ Transfer into LC-MS vial
  - ↓ Co-wash by 50  $\mu$ L 50% MeOH
  - ↓ Transfer into LC-MS vial
- 100  $\mu$ L permethylated sample in 50% MeOH
- ↓ Storage at -20°C

✓ Permethylation Kit is available from Ludger Ltd., and its price is ¥330,000 for 96 tests.