

## Protocol 7: Enrichment of Glycopeptides from Tryptic Mtb Digests

### Purpose:

This protocol describes steps to enrich glycopeptides from crude tryptic digests of guanidinium thiocyanate lysed Mtb using Concanavilin A (Con-A) affinity resin.

### Materials:

Tryptic digest from 200 µg of protein  
10 kDa molecular-weight cutoff filter (Microcon-10, Millipore)  
Con-A resin slurry (Sigma, cat # C7555)  
Micro-spin column (Pierce, product # 69705)  
Wide-bore pipet tip (Axygen)  
Vacuum-manifold solid-phase extraction unit (Waters)  
Con-A binding/wash buffer (Pierce, product # 89804)  
Elution buffer (1 M methyl- $\alpha$ -D-mannopyranoside in water)  
Digestion buffer (50 mM ammonium bicarbonate)  
Eppendorf microcentrifuge 1.5-mL, low protein binding (VWR, cat # 80077-232)

### Process:

1. The tryptic digest from approximately 200 µg of protein in 200 µL of Digestion buffer is loaded into the spin cup of a 10 kDa molecular-weight cutoff filter and spun at room temperature for 60 minutes at 10,000g. This step removes active trypsin from the sample.
2. The filtrate is collected and used for the Con-A enrichment.
3. A 200-µL aliquot of Con-A resin slurry is transferred into a Micro-spin column using a wide-bore pipet tip. The micro-spin column is attached to a vacuum-manifold solid-phase extraction unit and the storage buffer is removed by vacuum application.
4. The Con-A resin is then washed 1X with Con-A binding/wash buffer followed by washing 2X with 100 µL of 50 mM ammonium bicarbonate.
5. The micro-spin column containing the washed resin is then removed from the manifold, the bottom of the unit is plugged and the unit is placed inside a 1.5-mL Eppendorf tube.
6. The sample from step 2 is then applied to the resin, the top is capped and the unit is gently mixed for 30 minutes at room temperature.
7. Collection tubes are placed inside the vacuum manifold and the micro-spin unit is re-attached and the unbound material is removed by applying the vacuum. The flow-through, glycopeptide depleted material can be saved for other applications.
8. The resin is next washed with ammonium bicarbonate (1X, 200 µL) and then water (2X, 200 µL). The first two washes are removed by applying the vacuum and the third and final wash is removed by spinning the spin columns in a microcentrifuge (2 minutes at 1000 g).
9. The bottom of the column is plugged again and the unit is placed into a low-protein-binding Eppendorf tube.
10. The glycopeptides are then eluted from the resin by adding 100 µL of Elution buffer and gently mixing for 30 minutes at room temperature.
11. The eluted material is collected by centrifugation (2 minutes at 1000 g) and then transferred into an autosampler vial for analysis.

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