SOP PP019.4
Modified 5/12/15 by MCL

Preparation of Con-A Reactive CFP's

Materials and Reagents:

1. Protein Sample
2. Concanavalin A-Sepharose 4B resin (Sigma)
3. Bio-Rad Econo Pump Chromatography system
4. Bio-Rad open column
5. ConA Binding buffer: 50mM KH_2PO_4, 500mM NaCl, 1mM each of $MgCl_2$, $CaCl_2$, $MnCl_2$ and DTT (note 1)
6. ConA Elution Buffer: ConA Binding buffer with 1M Methyl α-D-mannopyranoside (note 2)
7. Milli-Q water
8. Benchtop Centrifuge
9. Centricon-70 with 10,000 MWCO membrane
10. Ammonium bicarbonate

Protocol:

1._____ Resuspend the lyophilized protein sample in ConA Binding Buffer at a concentration of 1 mg/ml (note 3).

2._____ Calculate the amount of ConA resin needed give a ratio of 1 ml resin / 2-15 mg protein (note 4).

3._____ Add the ConA resin to an open column (note 5).

4._____ Before connecting the column to the pump, prime the lines first into water, then prime line "B" in ConA Elution buffer, followed by line "A" in ConA Binding buffer.

5._____ Equilibrate the resin by passing 3 column volumes of Con-A binding buffer through the column at 2 ml/min.

6._____ Load the sample into the column at flow rate of 1 ml/min, making certain to catch the eluent.

7._____ Pass the eluent through the column again. Collect and label as ConA Flow Thru.

8._____ To wash unbound material from the resin, pump three column volumes of Con-A buffer through the column at 1.5 ml/min. Collect and label as ConA Wash.

9._____ Elute bound material by pumping three column volumes of ConA elution buffer through the column at 1.5 ml/min into a fresh plastic container. This is the mannosylated protein fraction. Collect and label as ConA Bound.

10._____ Transfer fractions to centricon and centrifuge at 3000 rpm to concentrate down to ~2 ml.

11._____ Fill the centricon with 10mM ammonium bicarbonate and concentrate down.

12._____ Repeat step 11 two more times for a total of three washes.

13._____ Harvest sample from the centricon per product instructions and transfer to a sterile plastic container.

14._____ Run BCA (SOP:SP003) and SDS-PAGE (SOP:SP007 & SOP:SP012)

Notes:

1. Con A Buffer:
   To 80 ml of Milli-Q water stirring on a stirplate, add the following:

KH_2PO_4	0.69 g
NaCl	2.92 g
$MgCl_2 \cdot 6H_2O$	20.3 mg
$CaCl_2 \cdot 2H_2O$	14.7 mg
$MnCl_2 \cdot 4H_2O$	19.8 mg
DTT	15.4 mg

   After all reagents have gone into solution, add NaOH dropwise until the pH is 5.7. Transfer to a graduated cylinder and bring to final volume of 100 ml with Milli-Q water.
2. Con A Elution Buffer: Dissolve all the above reagents in 70 ml of Milli-Q water plus Methyl α-D-mannopyranoside 19.42 g. Titrate to pH = 5.7 and bring to final volume of 100 ml.
3. Some samples will not go into solution readily. Bath sonication may help suspend samples. Alternatively, samples may be exchanged into ConA binding buffer in lieu of being lyophilized and resuspended.
4. For partially purified samples (for example, phenyl-sepharose flow thru from Ag85 purification for Mpt32 purification) 5 mg protein/ml resin should be used. For crude samples such as CFP, up to 10-15 mg of protein/ml can be loaded. Other proteins such as 38kDa (PhoS1) should be loaded at 2 mg/ml of resin (see SOP: PP024, as the procedure for ConA purification of 38kDa is different than that listed here).
5. Note that the amount to ConA slurry needed is approximately 1.4 ml per 1 ml of desired packed column volume (*example: a 10 ml column will require 14 ml of ConA slurry*).