

SOP PP019.1
Modified 12-4-06

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. ____ Add volume of Con A buffer to lyophilized protein sample sufficient to yield a protein concentration of 1 mg/ml (note 3).
2. ____ Into an open column, pour a volume of Con-A resin which will give a ratio of 1 ml resin: 2-15 mg protein (note 4).
3. ____ Equilibrate the resin by passing 3 column volumes of Con-A binding buffer through the column at 1 ml/min.
4. ____ Load the sample into the column at flow rate of 1 ml/minute, making certain to catch the eluent.
5. ____ Pass the eluent through the column again. Collect and label as ConA Flow Thru.
6. ____ To wash unbound material from the resin, pump three column volumes of Con-A buffer through the column at 1 ml/min. Collect and label as ConA Wash.
7. ____ Elute bound material by pumping three column volumes of ConA elution buffer through the column at 1 ml/min into a fresh plastic container. This is the mannosylated protein fraction. Collect and label as ConA Bound.
8. ____ Transfer fractions to centricon and centrifuge at 3000rpm to concentrate down to ~2 ml.
9. ____ Fill the centricon with 10mM ammonium bicarbonate and concentrate down.
10. ____ Repeat step 9 two more times for a total of three washes.
11. ____ Recover sample from the centricon and transfer to a sterile plastic container.
12. ____ Run BCA and SDS-PAGE

Notes:

1. Con A Buffer:

To 80 ml of Milli-Q water stirring on a stirplate, add the following:

| | |
|--------------------------------------|---------|
| KH ₂ PO ₄ | 0.69 g |
| NaCl | 2.92 g |
| MgCl ₂ ·6H ₂ O | 20.3 mg |
| CaCl ₂ ·2H ₂ O | 14.7 mg |
| MnCl ₂ ·4H ₂ O | 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 Mill-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 38kDa purification from 70% ammonium sulfate cut, use 2 mg protein/ml resin. For phenyl-sepharose flow thru from Ag85 purification (for 45kDa purification) 5 mg protein/ml resin should be used. For crude samples such as CFP, up to 10-15 mg of protein can be loaded.