SOP: PP022.6
Modified 10/1/2015 MCL

Purification of MPT32 Protocol

Materials and Reagents:

1. Mannosylated CFP pool (note 1)
2. UV HPLC with Empower software
3. 20 ml C4 reverse phase column
4. Buffer A: 20mM ammonium bicarbonate and 1mM DTT (note 2)
5. Buffer B: 20mM ammonium bicarbonate in 80% acetonitrile (ACN) (note 3)
6. 12 x 75 mm disposable culture tubes
7. Dialysis tubing, 12-14000 MWCO
8. Dialysis tank
9. Ammonium bicarbonate

Protocol:

1._____ Prime HPLC lines with the appropriate buffers and water (Line A: buffer A, etc).

2._____ Attach the C4 reverse phase (RP) column to the HPLC and wash with water for ~ 1 hour at 1 ml/min to remove storage buffer (note 4)

3._____ Equilibrate the column in buffer A for ~ 1 hour at 1 ml/min.

4._____ Resuspend mannosylated CFP starting material in buffer A at a concentration of 1mg/ml.

5._____ Once the column is equilibrated, inject the protein sample into the HPLC (note 5).

6._____ Run the "large C4" program on Empower (note 6).

7._____ Draw 10 ml of the filtered protein solution into a 10 ml syringe. Free the syringe of any bubbles by gently tapping it on a hard surface (the bubbles should move to the surface). Expel the bubbles by pushing up on the plunger. Attach the Waters injection needle and expel some of the liquid through the needle. This is to make sure that there are not any air bubbles preceding the liquid.

8._____ Move the HPLC injection lever to "load", insert the needle into the injection lever and expel the liquid by pushing on the plunger. After all the liquid has been dispensed, remove the needle from the injection lever and move the lever to "inject".

9._____ If more injections are required, wait 11 minutes, then repeat injection (steps 7-8). Repeat as many times as necessary to inject all material, being sure to collect the flow through from the injection and wash.

10._____ Before making the final injection, click on the inject icon on the Empower software. Collect fractions in 5 mL polypropylene tubes.

11._____ After the method is complete, flush C4 with filtered water for 2 column volumes to ensure salt removal and store in 50% methanol

12._____ Aliquot 10 µl of each fraction into a 0.65 mL eppendorf tube and dry in the Savant to remove ACN.

13._____ Resuspend each fraction in 10 µl 10mM ammonium bicarbonate, and run each fraction on a gel (SOP: SP006) and silver stain (SOP: SP012)

14._____ Determine with fractions contain the 45kDa antigen and dry them down on the Savant for ~ 1-2 hours to remove the ACN.

15._____ Pool the fractions together and dialyze against 10mM ammonium bicarbonate and 1mM DTT at 4°C, with at least 3 exchanges of buffer 4-24 hours apart.

16._____ Collect pool and concentrate in a 30k MWCO amicon spin column, or lyophilize and resuspend in a minimal amount of 10 mM ammonium bicarbonate when dry.

17._____ Perform a BCA assay (SOP: SP003), SDS-PAGE, and Western Blot (SOP: SP011) using the CS-93 antibody, for QC

18._____ Make aliquots (default quantity is 0.5 mg) and lyophilize, then store at -80°C.

Notes:

1. CFP should be passed over a ConA column, per SOP PP019. Alternatively, the CFP can be passed over a phenyl sepharose column first for recovery of Ag85, then over the ConA column, however, be sure that DTT is kept in the buffers at all times and that the flow through is processed quickly to ensure that the Mpt32 does not break down.
2. Buffer A: 500 ml
    - 790.56 mg ammonium bicarbonate
    - 77.124 mg DTT
    - QS with Milli-Q H_2O
3. Buffer B: 500 ml *Make in glass container*
    - 790.56 mg ammonium bicarbonate
    - 400 ml acetonitrile
    - QS with Milli-Q H_2O
4. Once the column is attached, start washing at 0.5 ml/min, then gradually move up to 1 ml/min over the course of ~ 15 min
5. Before using the HPLC and Empower HPLC program, read the HPLC SOP: SP025 or have lab personnel trained in the use of the HPLC assist you in setting up the liquid chromatography.
6. Run the following program at 1 ml/min:

20 min wait	100% buffer A
40 min gradient	100% A → 40% A/ 60% B
25 min hold	40% A/ 60% B
20 min hold	100% buffer B

    Collect flow thru and elution wash. Collect 2 minute fractions starting at the gradient through the 100% hold. Adjust times as needed to bigger or smaller columns.