PSMA Protein Purification Protocol

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(1) Protein is obtained from Protein Expression Facility at Caltech. Usually only 1-2 L are ordered since the PSMA expression level is high (>10 mg/L). The supernatants are stored at 4ºC.

(2) Concentrate and Exchange

a) prepare 10kDa cut-off membrane with Amicon 2L concentrator, washing it and pre-equilibrating with 500 mL buffer (50 mM Tris pH=7.5, 150 mM NaCl)
b) add 0.2 or 0.45 μm-filtered protein to reservoir, follow concentrator/membrane instructions keeping pressure below 20 psi
c) concentrate protein to ~200 mL at RT
d) add 1 L buffer (50 mM Tris pH=7.5, 150 mM NaCl)
e) repeat steps b)+c) for a total of 3 times (this can also be done by diafiltration for total of 3 L of buffer)
f) concentrate to 200 mL
g) drain concentrator into 1L bottle
h) wash concentrator with ~500 mL buffer to get all protein out into 1L bottle
i) adjust the ~700 mL of protein so that it is 50 mM Tris pH=7.5, 300 mM NaCl, 10% glycerol, 10mM imidazole (add azide if desired)
j) mix well
k) 0.2 μm filter the protein solution
l) wash concentrator for next user

(3) Nickel Column

a) prepare Ni column per Qiagen instructions
b) use minimal Ni-NTA beads (a mL of beads can bind several mgs of protein)
c) load O/N at 4ºC at rate less than or equal to 1 mL/min
d) hook column to U/V detector at RT
e) wash column until baseline is flat at rate less than or equal to 1 mL/min (wash buffer = 50 mM Tris pH=7.5, 300 mM NaCl, 10 mM imidazole, 10% glycerol, azide if desired)
f) elute column at 2 mL/min (elution buffer = 50 mM Tris pH=7.5, 300 mM NaCl, 250 mM imidazole, 10% glycerol, azide if desired)

(4) SEC

a) concentrate protein at ~10 ºC using amicon concentrator (15 mL size, 10kD membrane)
    -I try to keep protein at less than 8 mg/mL so I will concentrate to 2-4 mL depending on how much supernatant I started with, expression level, etc
b) pre-equilibrate column (S200 16/60) with wash buffer (50 mM Tris pH=7.5, 150 mM NaCl)
c) I do 2 mL or smaller injections onto this column so sometimes I will do several runs if I have a lot of protein
d) run column at 1 mL/min and collect fractions (1.5 mL/fraction) using Akta program
e) run gel of fractions containing protein (7.5 % SDS-Page Reducing conditions; PSMA runs near 97.4 MW marker)
f) the dimeric PSMA fractions are what I use for crystallography applications

(5) If needed: Factor Xa cleavage will remove the N-terminal 6x-His-tag

a) if ~10 mL of protein from the sizing column use 2.5 mL water, 20 μL $CaCl_2$ (from 1M stock) and 40 μL of Factor Xa. (this protocol was from another protein in the lab and I didn't optimize it; the Factor Xa instructions for their assay conditions are 1μg Factor Xa to 50 μg of their test protein. Their reaction was in 50 μL, 20 mM Tris-HCl, 100 mM NaCl, 2 mM $CaCl_2$ (pH=8.0))

b) this reaction is allowed to go for 24 hours at 4°C on a very slowly turning rotator (very fast rotation leads to extra ppt)

c) the protein is purified via Ni-NTA (removes any uncleaved or free tags) and the SEC as above to separate Factor Xa from PSMA. (There is a Factor Xa you can buy that binds to Ni-NTA but I haven't tried that)