SRF-Bio-ASAP XXD2VNS2BA-c003 expression and purification

PAGE23-01988

Author: **Wang, Korvus**
Date Started: **2023-Nov-30**
Experiment Started: 2023-Dec-04
Projects: **Expression**;**Purification**;**ASAP**
Related Pages: **PAGE23-00729**
Referenced by:
Tags:

Info

Expression ID: XX01D2VNS2BA-e010
Purification ID: XX01D2VNS2BA-p007

XX01D2VNS2BA-c003

MHHHHHHSSMSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYI
ADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDA
LDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSGGGSENLYFQ//SMADLELERAA
DVKWEDQAEISGSSPILSITISEDGSMSIKNEEEEQTL

WITH TAG:
MW: 33132.88
E(red): 49850
PI: 5.32

Cleaved NS2B:
SMADLELERAADVKWEDQAEISGSSPILSITISEDGSMSIKNEEEEQTL

MW: 5383.85
E(red): 5500
PI: 3.85

Coexpression with NS3
MLEDGAYRIKQKGILGYSQIGAGVYKEGTFHTMWHVTRGAVLMHKGKRIEPSWADVKKDLISYGGGWKLEGEWKEGEEVQV
LALEPGKNPRAVQTKPGLFKTNAGTIGAVSLDFSPGTSGSPIIDKKGKVVGLYGNGVVTRSGAYVSAIAQTEKSIEDNPEIEDDI
FR

cleaved NS2B+NS3
MW: 23721.6
E(red): 36440
Final A280: 35.0
Final conc: 10-20 mg/mL

Title missing - double click to edit

Expression
10mL ON -> 100mL ON -> 6L 1xTB (made with Formedium powder) + Kan
Grown until OD~2. Induced with 0.5mM IPTG. 18oC 180RPM ON.
Harvested at 4000xg

60g +90g pellet total

IMAC

Buffers
Base buffer - 50mM HEPES pH7.5, 300mM NaCl, 5% glycerol, 0.5mM TCEP
Lysis buffer - base buffer
Wash buffer 1 - base buffer + 30mM imidazole
Wash buffer 2 - base buffer + 50mM imidazole
Elution Buffer (IMAC) - base buffer + 500mM imidazole
Gel Filtration Buffer (SEC) **-** 50mM HEPES pH7.5, 300mM NaCl, 5% glycerol, 0.5mM TCEP

5/12/2023

Cell Lysis

1. Add lysis buffer to pellet until total volume is 1000mL. Supplement with 1:4000 dilution of benzonase, 0.5mg/mL lysozyme, 1mM MgCl, 1 PIC tablets. Mix lysis mixture in cold room until homogenous.
2. Sonicated in cold room at 40% amplitude for a total of 7-minute sonication time (4 seconds on 12 seconds off) with thick probe
3. Clarified lysate by centrifugation at 38,000xg , 4°C for 1 hour.

IMAC

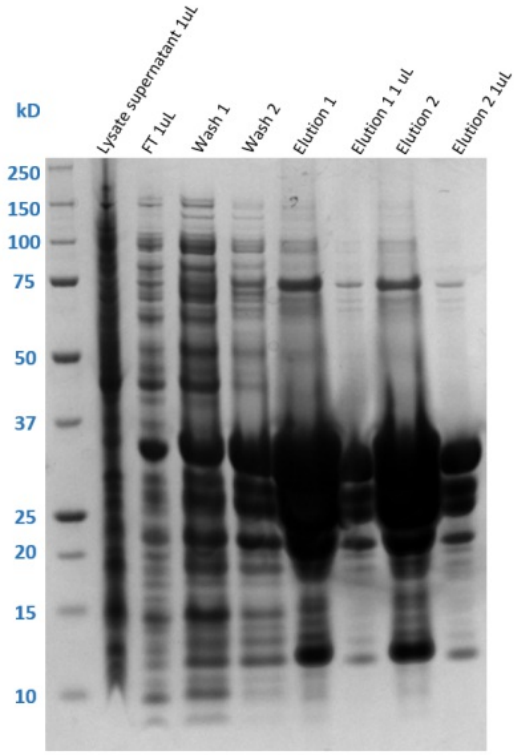
1. Wash and equlibrate 5mL bed volume of Ni Sepharose 6 FastFlow resin (regenerated) on gravity flow column, first with distilled water, then with base buffer.
2. Incubate clarified lysate with equilibrated resin for 30min in cold room.
3. Wash resin with 50mL each of wash buffer 1 and 2
4. Elute with 7.5mL elution buffer, 10min incubation. 2 elutions carried out.

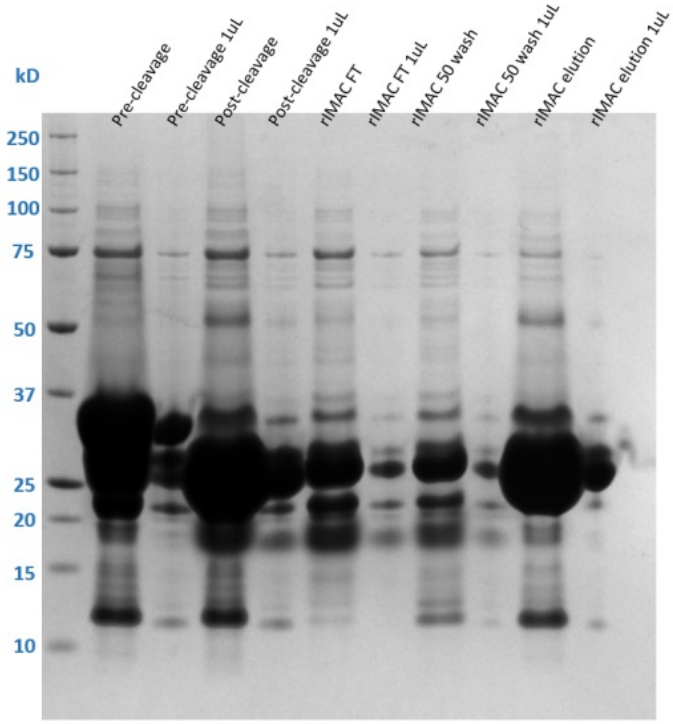
Desalting and cleavage
Both elutions pooled and desalted with HiLoad 26/10 desalting column into base buffer.
200uL of His-TEV (7.4mg/mL) is added to desalted sample and incubated in cold room overnight.

6/12/2023

rIMAC
IMAC resin was washed with ~100mL base buffer.
Cleavage mixture passed over IMAC resin once.
25mL wash buffer 2 (50imi) is used to wash the rIMAC resin.
Elution carried out with 10mL elution buffer.

IMAC and cleavage



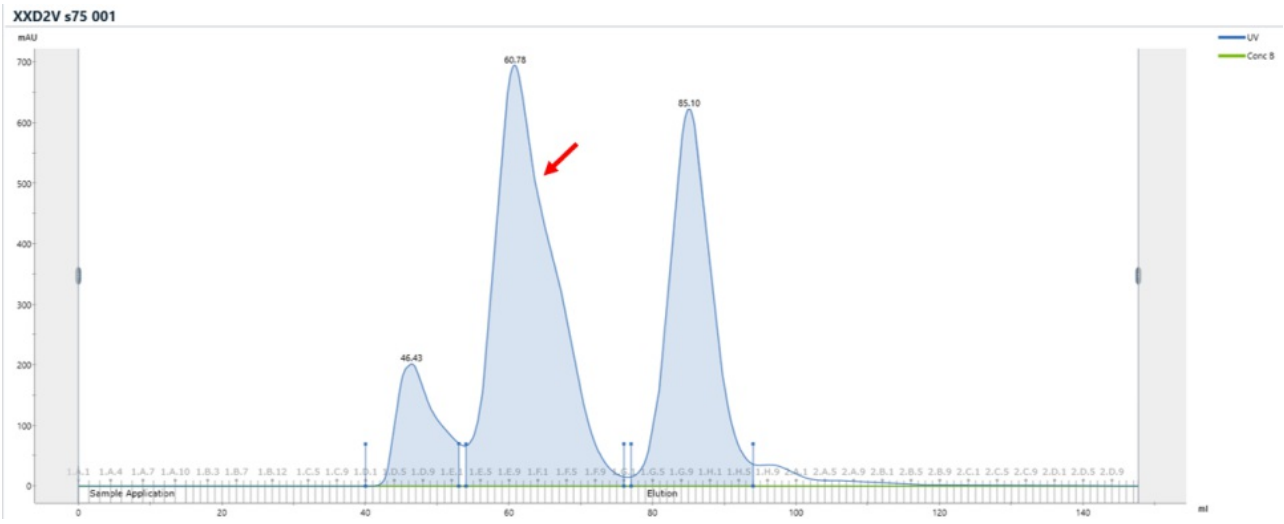


Gel filtration

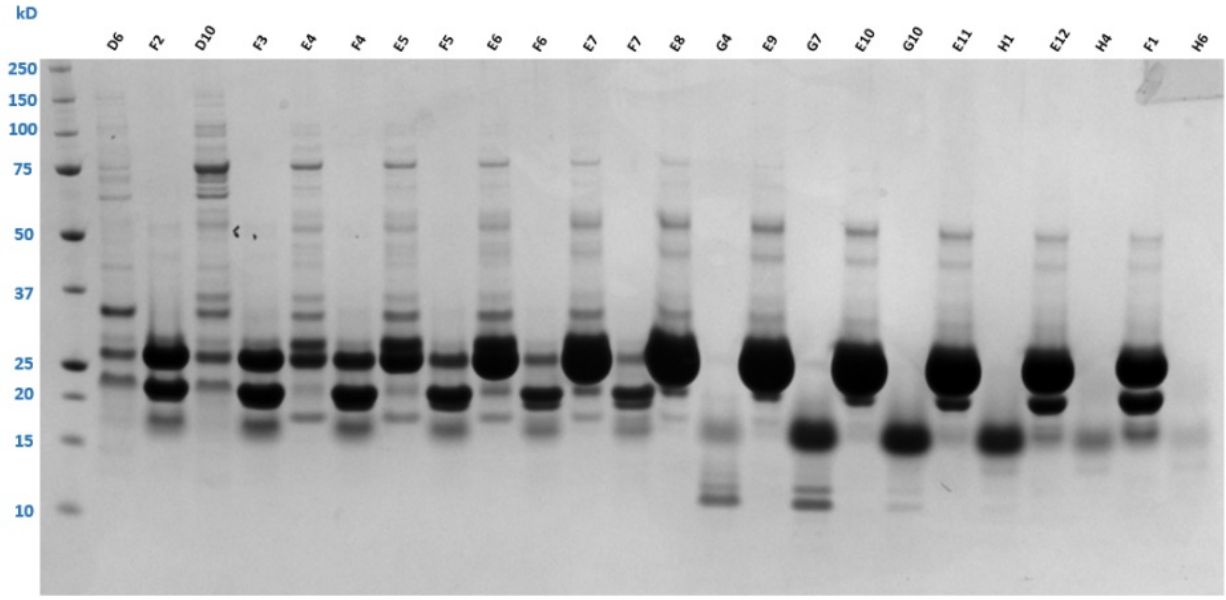
6/12/2023

rIMAC and 50imi wash was pooled and concentrated in Amicon 3kDa MWCO concentrators to ~4mL to be loaded onto s75 16/600 column.

s75 chromatogram



s75 fractions



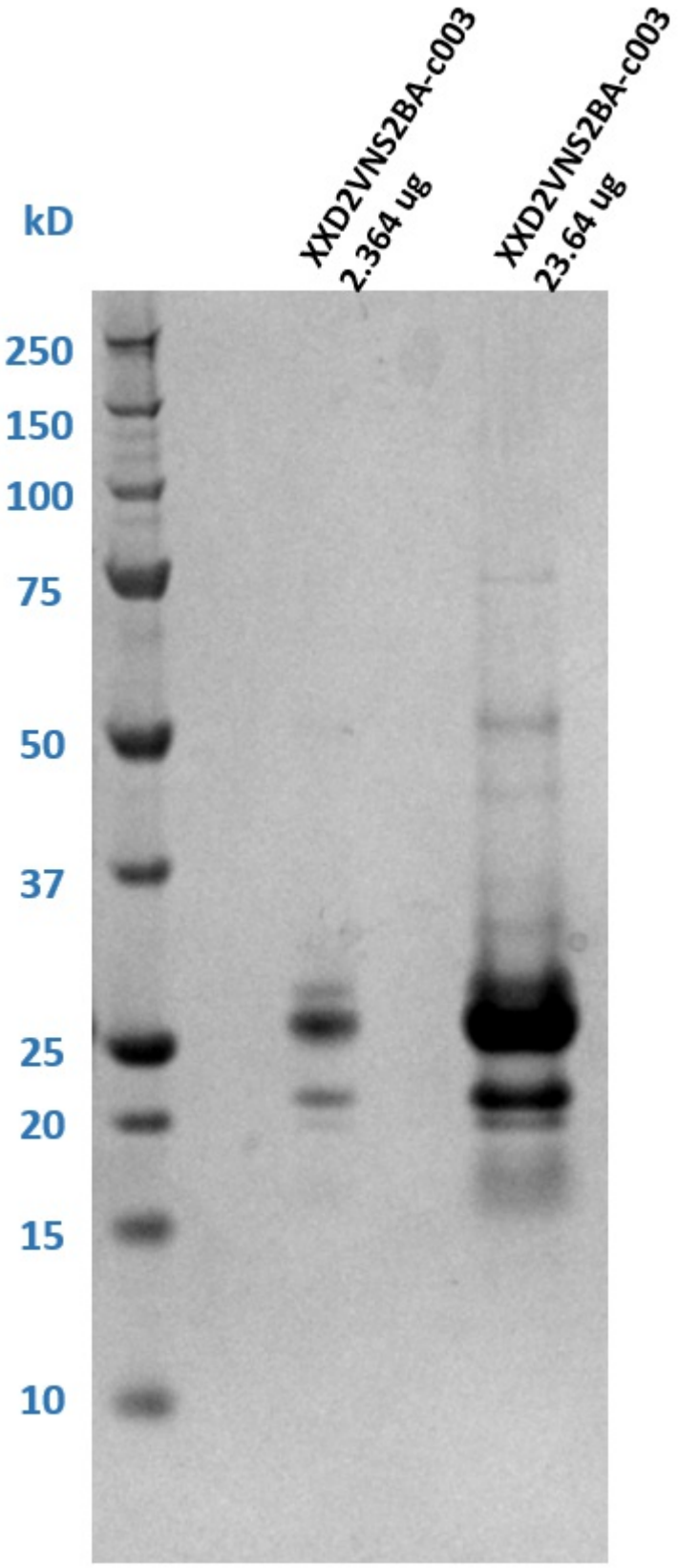
Final sample

7/12/2023

Fraction E5-F7 were pooled and concentrated in Amicom 10kDa MWCO concentrators.

Final concentration: 23.64 mg/mL, 12x60uL + ~10uL
Final yield: 17.26mg

Final sample



Final sample concentration


Protein UV
Absorbance (10 mm)
39
35
30
25
20
15
10
5
-0
200
220
240
260
280
300
320
340
360
380
400
Wavelength
Concentration
23.636
mg/ml
A260/A280
0.514
A280 (10 mm path)
36.44
enter sample name
BLANK
AUTO SAMPLE
SAMPLE