Enterovirus coxsackievirus A16 2A protease (EV2A) protease ex pression and purification round 7

PAGE23-01675

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Date Started: **2023-Oct-10**
Experiment Started:
Projects: **Expression**;**Purification**;**ASAP**
Related Pages: **PAGE23-00884**
Referenced by:
Tags:

Title missing - double click to edit

Same expression conditions - copy from last time

split into 3x2L. around 50g each.

EV2A

Clone ID: A71EV2A-k005 (same as last)
Expression ID: A71EV2A-e009
Purification ID: A71EV2A-p008
(2/6L of e009)

Note: ID does not refelct actual identity of the product - Enterovirus coxsackievirus A16 2A protease

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2L purification

Base buffer - 50mM HEPES pH7.5, 500mM NaCl, 5% glycerol, 0.5mM TCEP
Lysis buffer - base buffer +20mM imidazole
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) - 25mM HEPES pH 7.5, 300mM NaCl, 5% glycerol, 0.5mM TCEP

Cell Lysis

1. Add lysis buffer to pellet until total volume is 200mL. Supplement with 1:4000 dilution of benzonase, 0.5mg/mL lysozyme, 2mM MgCl, 2 PIC tablets, 25mM imidazole. Mix lysis mixture in cold room until homogenous.
2. Sonicated in cold room at 40% amplitude for a total of 10-minute sonication time (2 seconds on 4 seconds off) with thick probe
3. Clarified lysate by centrifugation at 35,000xg , 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. Allowed lysis mix to flow through resin without incubation
3. Wash resin with 100mL lysis buffer, then 1 and 2 wash buffer 50mL each.

4. Elute with 7.5mL elution buffer, 10min incubation. 2 elutions carried out.

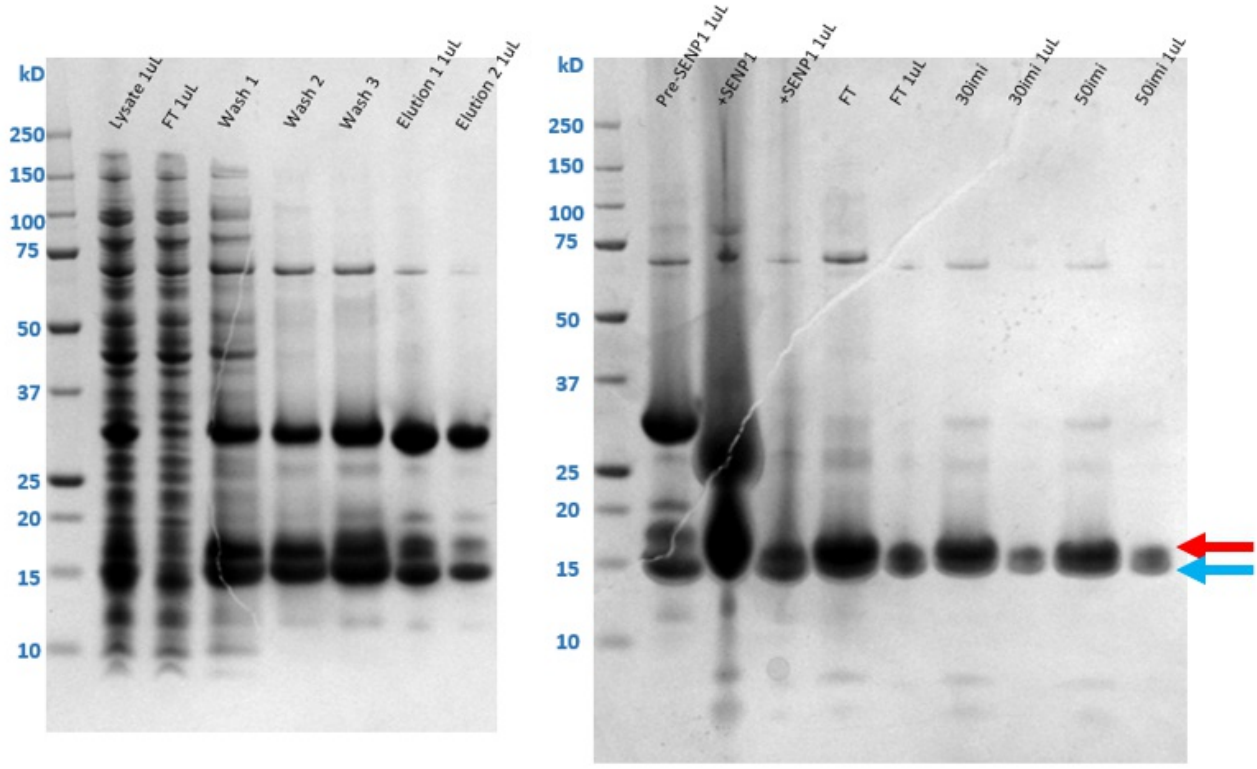
At 1OD=1mg/mL:
E1: 13.6 mg/mL
E2: 10.3 mg/mL

Desalting and cleavage
Desalting carried out with Sepharose 26/10 desalting column on ATKA.
After desalt: 7.26mg/mL, 15mL
His-SENP1 was added at 1:100 ratio and left to incubate in cold room overnight.

rIMAC
In morning, IMAC resin was washed with ~100mL base buffer.
Cleavage mixture passed over IMAC resin twice.

FT and 30imi wash pooled and concentrated in 10kDa MWCO concentrators.

IMAC and cleavage result

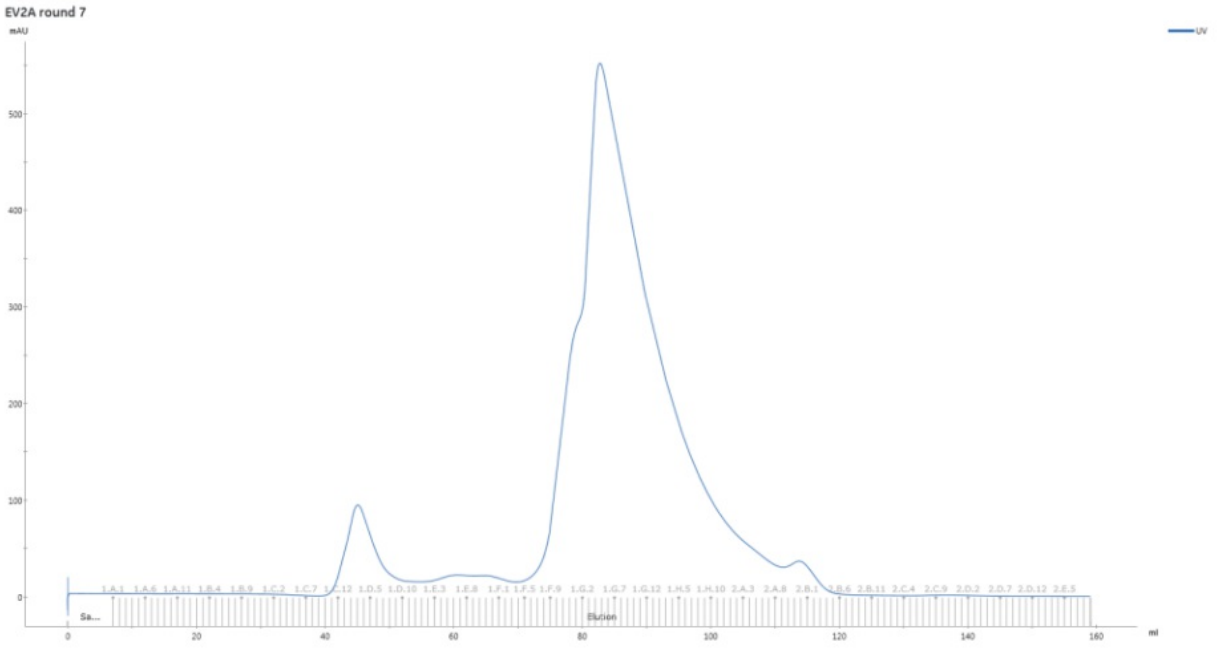


SEC

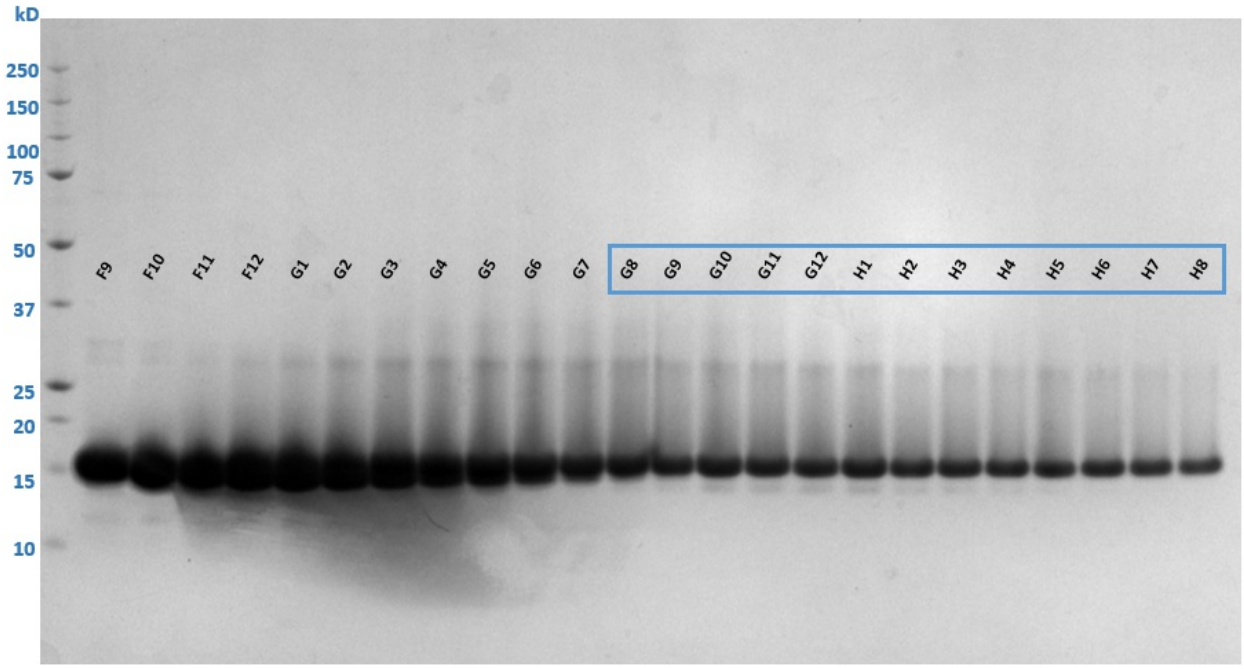
Red- EV2A
Blue- SUMO tag

RAN IN DIFFERENT SEC BUFFER AS LAST TIME

Chromatogram



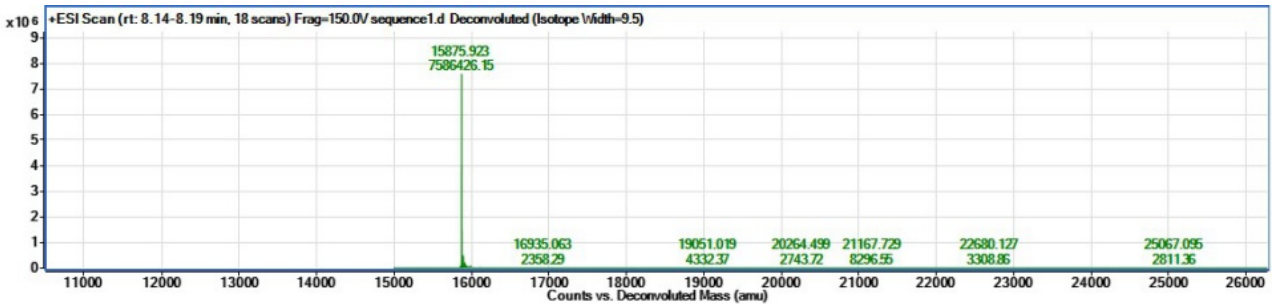
sec S75 16/60 result



Final

Fraction G8-G12 pooled and concentrated in 10kDa MWCO amicon concentrators.
Final concentration: 18.65 mg/mL, 22*20uL + ~10uL
Total yield: 8.39mg from 2L

MS


x10 6
+ESI Scan (rt: 8.14-8.19 min, 18 scans) Frag=150.0V sequence1.d Deconvoluted (Isotope Width=9.5)
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16935.063
2358.29
19051.019
4332.37
20264.499
2743.72
21167.729
8296.55
22680.127
3308.86
25067.095
2811.36
Counts vs. Deconvoluted Mass (amu)