AVIDD ASAP: EVD68 & EVA71 3C protease expression and purification

PAGE22-01976

Author: **Fairhead, Michael**
Date Started: **2022-Nov-21**
Experiment Started:
Projects: **Expression**;**Purification**;**ASAP**
Related Pages:
Referenced by: **PAGE23-00563**
Tags:

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D68EV3CPROA-c001/D68EV3CPROA-k001/D68EV3CPROA-e001/D68EV3CPROA-p001

MGPGFDFAQAIMKKNTVIARTEKGEFTMLGVYDRVAVIPTHASVGEIIYINDVETRVLDACALRDLTDTNLEITIVKLDRNQKFR
DIRHFLPRCEDDYNDAVLSVHTSKFPNMYIPVGQVTNYGFLNLGGTPTHRILMYNFPTRAGQCGGVVTTTGKVIGIHVGGNGA
QGFAAMLLHSYFTDTQKHHHHHH

21283.3 Da

10430 mM-1cm-1

A71EV3CPROA-c001/A71EV3CPROA-k001/A71EV3CPROA-e001/A71EV3CPROA-p001

MGPSLDFALSLLRRNIRQVQTDQGHFTMLGVRDRLAVLPRHSQPGKTIWVEHKLINILDAVELVDEQGVNLELTLVTLDTNEKF
RDITKFIPENISAASDATLVINTEHMPSMFVPVGDVVQYGFLNLSGKPTHRTMMYNFPTKAGQCGGVVTSVGKVIGIHIGGNG
RQGFCAGLKRSYFASEQLEHHHHHH

21331.5 Da

9970 mM-1 cm-1

From
https://doi.org/10.1107/S0907444913002862
The protein was stored in 25 mM Tris–HCl pH 8.0, 150 mM NaCl, 1 mM TCEP at 277 K.

crystallization example

The droplets were prepared by a 1:1 mix of the complex (12 mg/ml) and the reservoir solution containing 100 mM Tris-HCl pH 8.5, 25% PEG4000, and 0.8 M lithium chloride. The crystals were ready for data collection at 16 °C for about 1 week. (https://doi.org/10.1002/jmr.2551)

Score	Expect	Method	Identities	Positives	Gaps
214 bits(544)	4e-76	Compositional matrix adjust.	99/184(54%)	128/184(69%)	0/184(0%)

```
Query 1 MGPGFDFAQAIMKKNTVIARTEKGEFTMLGVYDRVAVIPTHASVGEIIYINDVETRVLDA 60
Sbjct 1 ...SL...LSLLRR.IRQVQ.DQ.H.....R..L..L.R.SQP.KT.WVEHKLINI... 60
```

```
Query 61 CALRDLTDTNLEITIVKLDRNQKFRDIRHFLPRCEDDYNDAVLSVHTSKFPNMYIPVGQV 120
Sbjct 61 VE.V.EQGV...L.L.T..T.E.....TK.I.ENISAAS..T.VIN.EHM.S.FV...D. 120

Query 121 TNYGFLNLGGTPTHRILMYNFPTRAGQCGGVVTTTGKVIGIHVGGNGAQGFAAMLLHSYF 180
Sbjct 121 VQ......S.K....TM......K.........SV.......I....R...C.G.KR... 180

Query 181 TDTQ 184
Sbjct 181 ASE. 184
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15 mL o/n in SOC + Kan 50

Use to inoculate 1L AIM-TB

grow 4h 37C/20h 17C 250 rpm shaking

Harvest 4000 g (grew 2L of each protease, around 100 g of cells total for each protease)

Lyse in base buffer + 1 % TX-100 and 0.5 mg/mL HEWL + 1 ug/mL benzonase + 30 mM Imidazole

Centrifuge 30,000g 1h 4C

Pour SN over 15 mL Ni Sepharose FF

Wash 3 x 100 mL Base Buffer + 30 mM Imidazole

Elute 3 x 30 mL Base Buffer + 30 mM Imidazole (protein predominantly in 1st 2 fractions)

D68EV3CPROA
Fraction 1 = 30 mL A280 of 9
Fraction 2 = 30 mL A280 of 6
Fraction 3 = 30 mL A280 of 1.3

A71EV3CPROA
Fraction 1 = 30 mL A280 of 3.4
Fraction 2 = 30 mL A280 of 3.1
Fraction 3 = 30 mL A280 of 1.2

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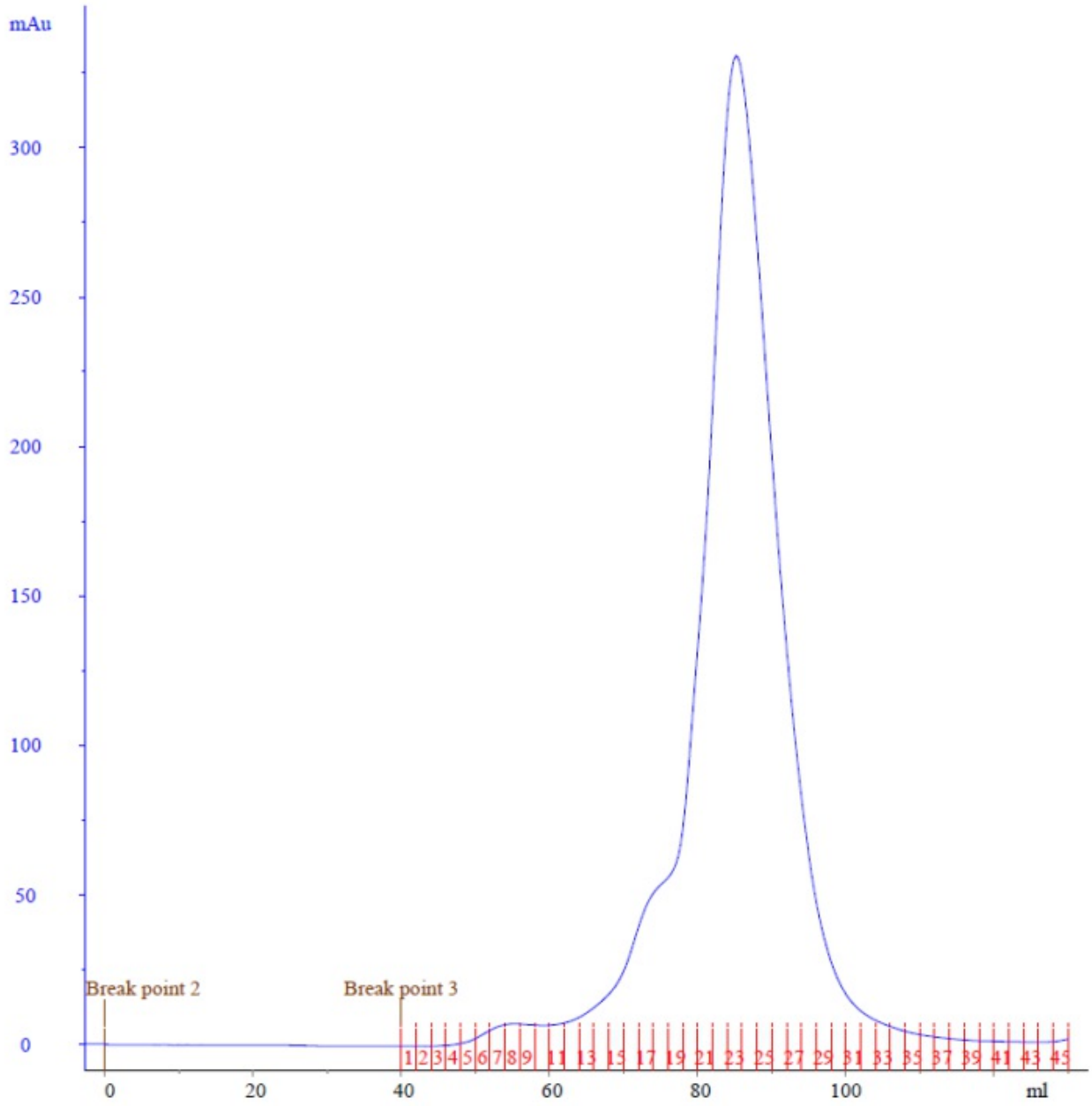
Dialyze o/n into base buffer + 0.5 mM TCEP

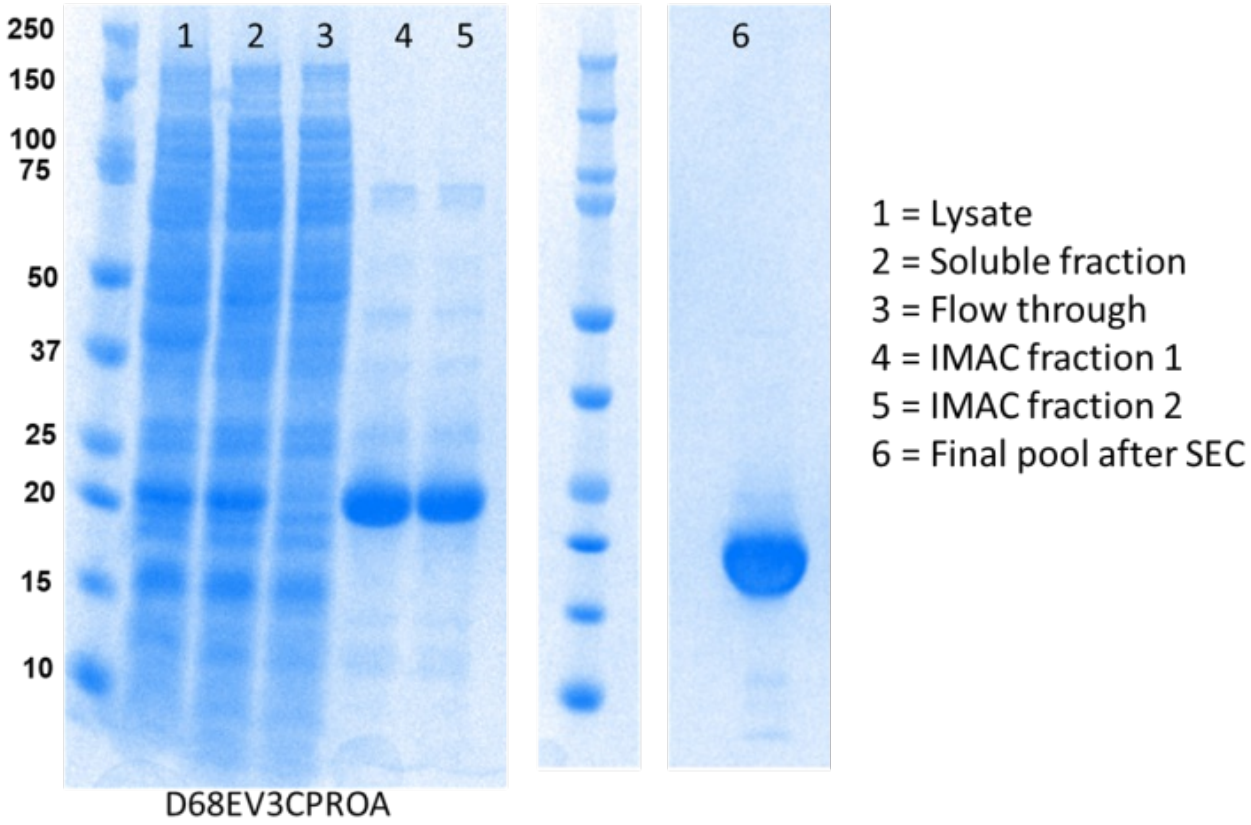
Very very slow to concentrate in both cases about 30 mL was the best I could do in both cases (about 10 mg/mL of EVD68 and 5 mg/mL for EV71)

Run 5 mL aliquots over 125 mL superose 12 pg column using base buffer + 0.5 mM TCEP as mobile phase

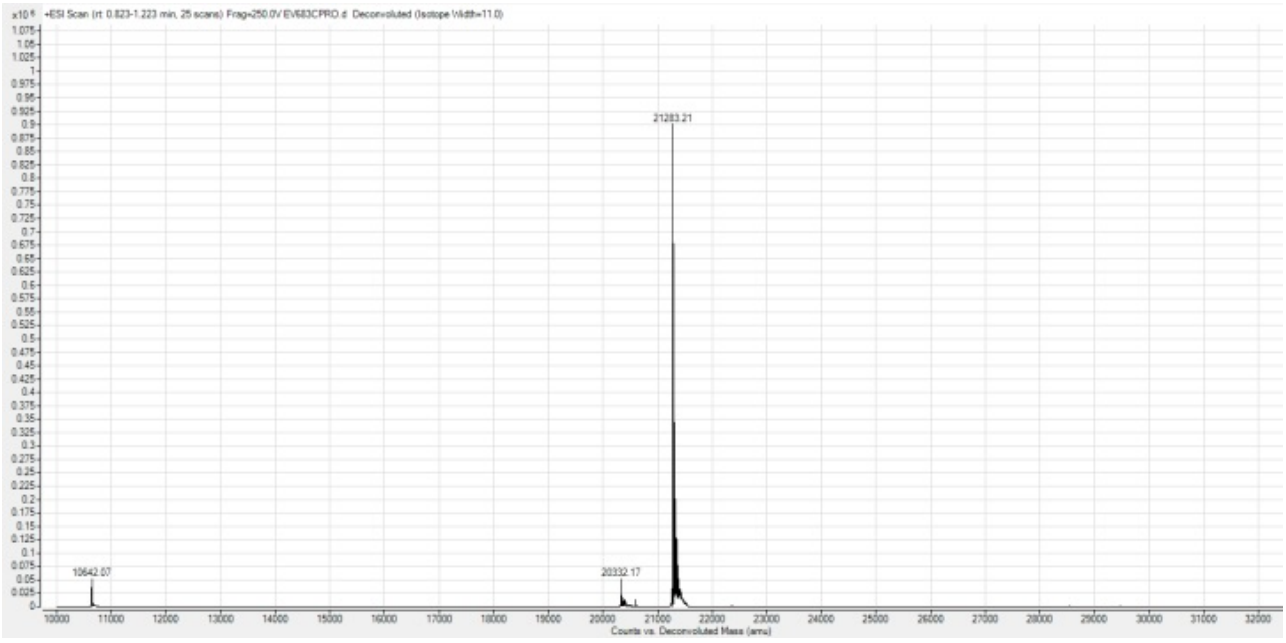
EVD68 3CL Protease superose 12 pg column



SDS PAGE D68EV3CPROA



D68EV3CPROA-p001 MS confirmation Z:\Agilent_SGC_QTOF\Rod\221124_MS424C



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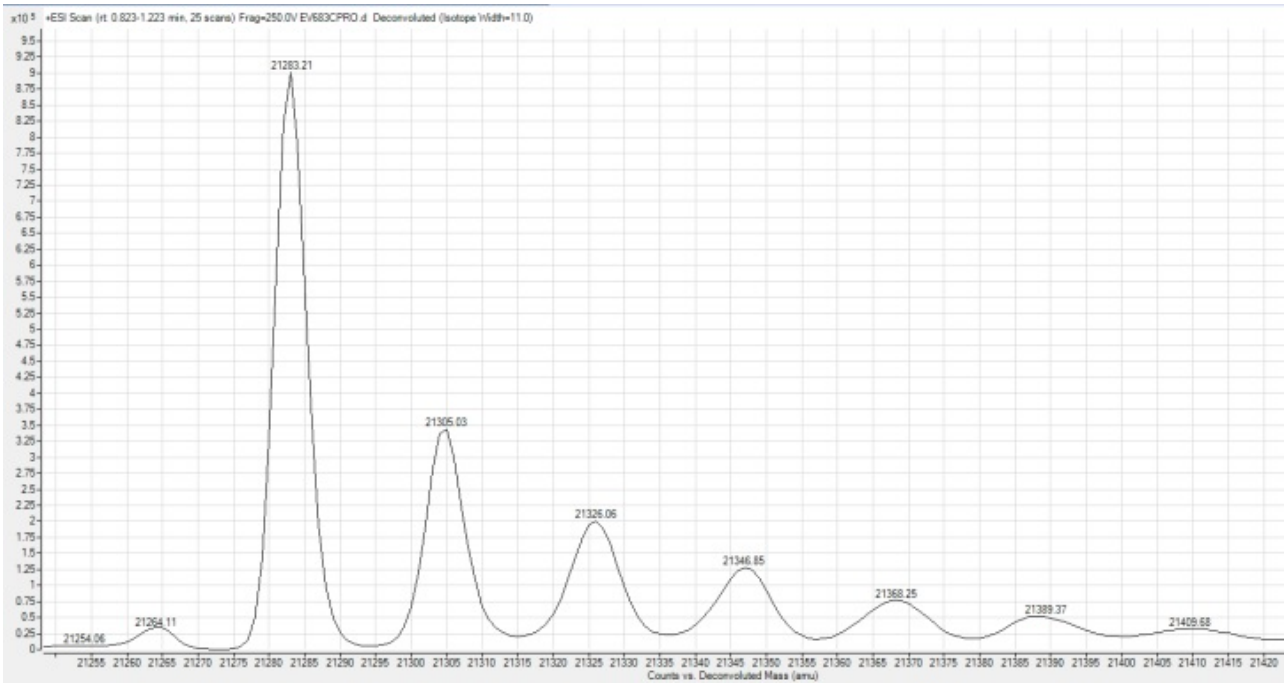

EVD68 3CL Protease

EVD68 pool concentrates ok to 8.2 (786 uM) 16.7 mg mL
just over 1.2 mL per run so about 20 mg can do 6 runs in total so about 120 mg from 2 L or 60 mg/L final
Did three runs

Mass-Spec shows MW 21283.21 Da observed versus 21283.3 Da expected

D68EV3CPROA-p001
1 mM 31 x 50 uL aliquots after making plates
300 nL drops in

CI084721 HCS
CI084722 BCS
CI084723 LFS
CI084724 MORPHEUS
CI084725 JCSG
CI084726 HIN

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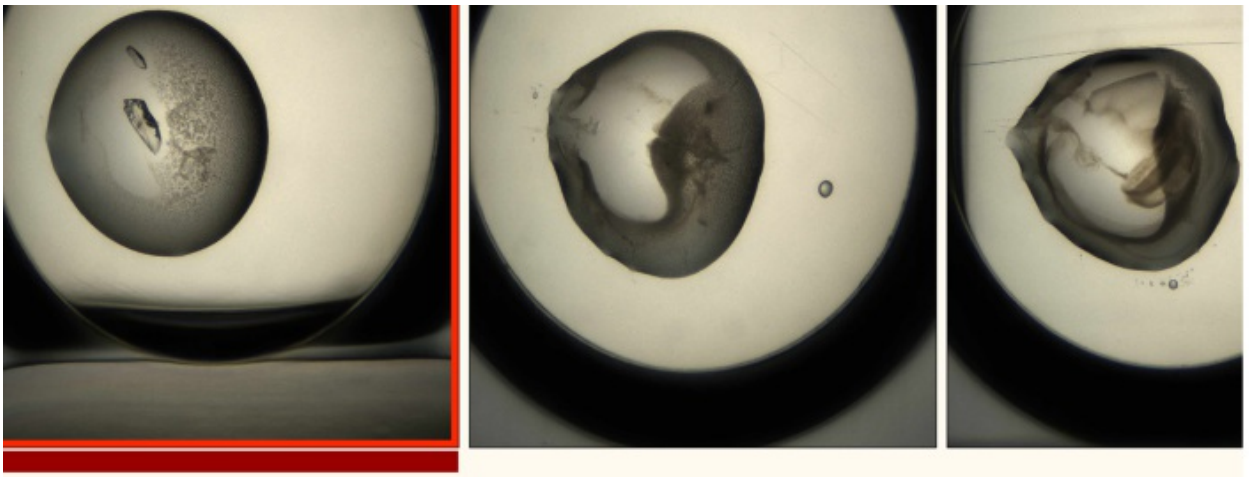
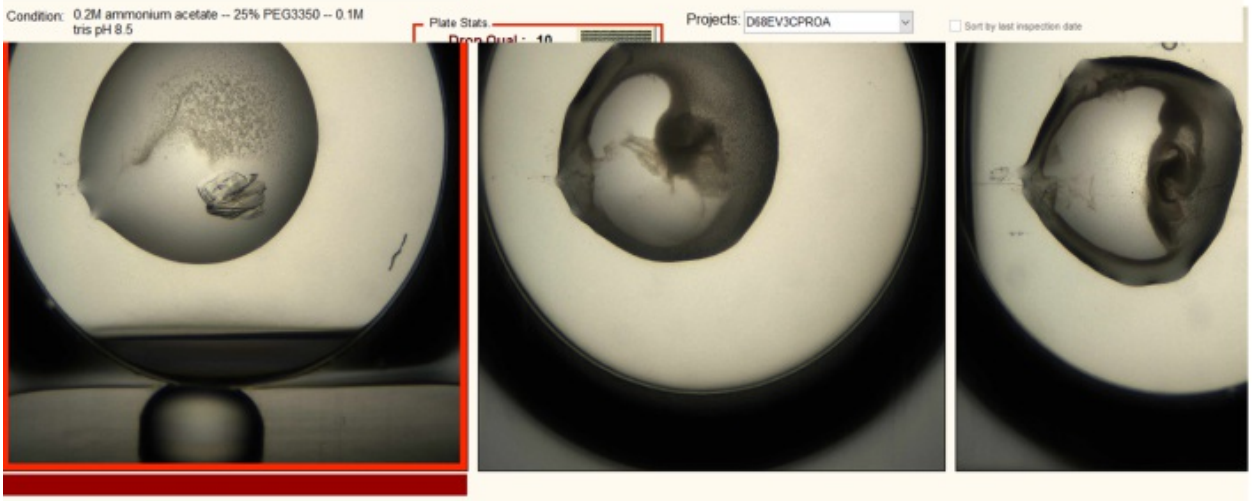
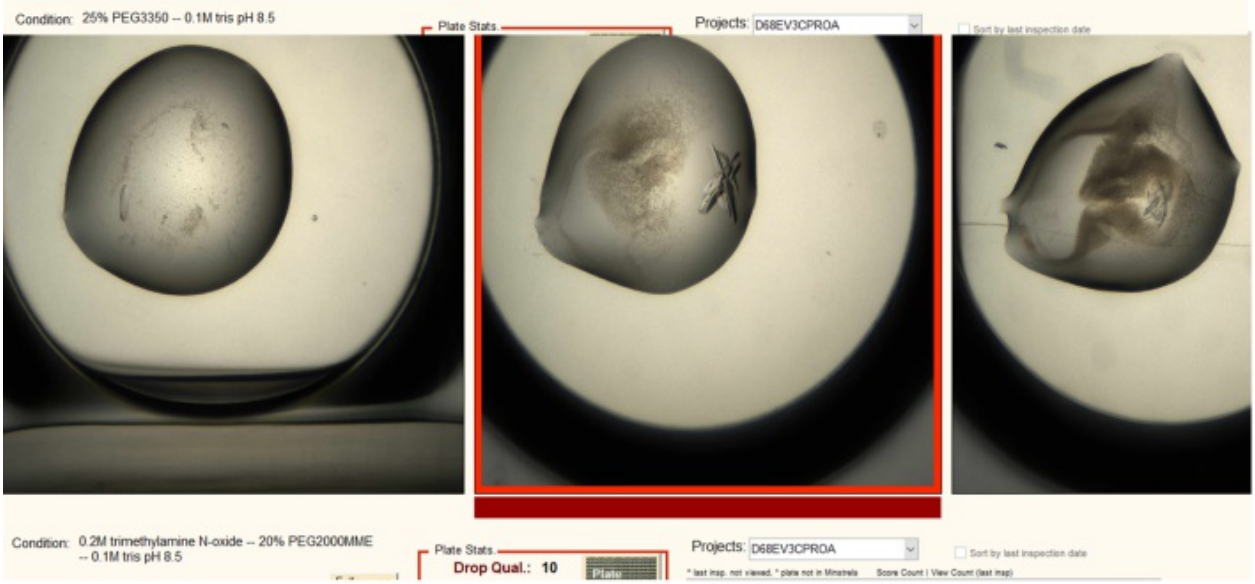
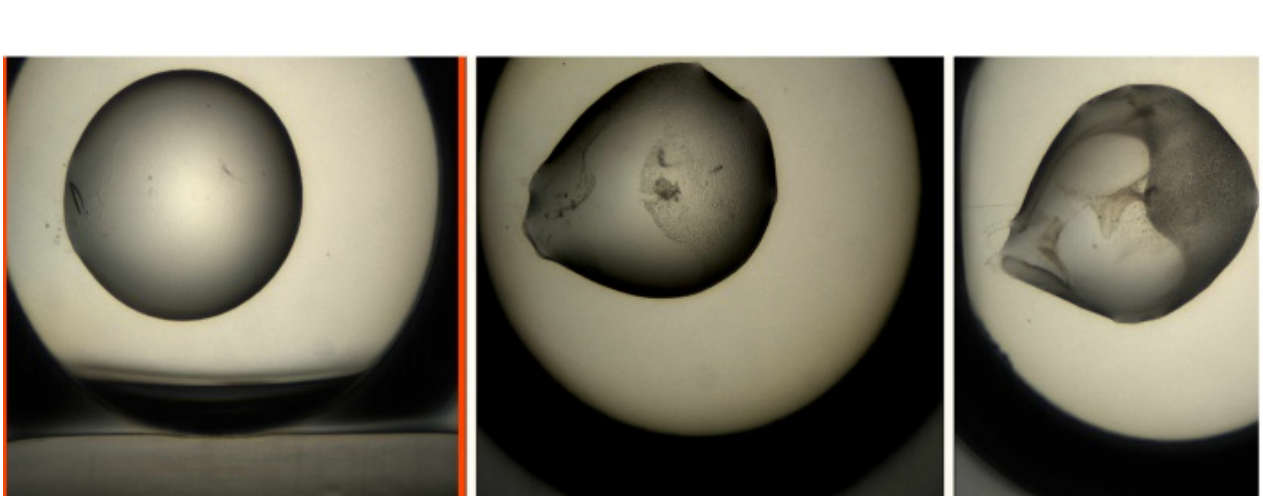
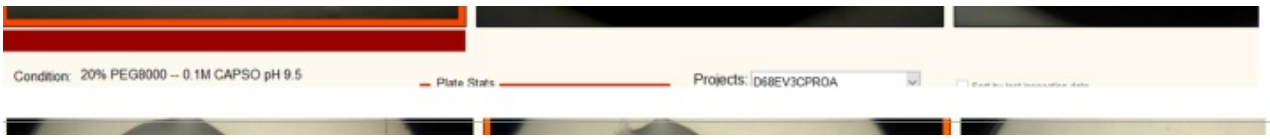


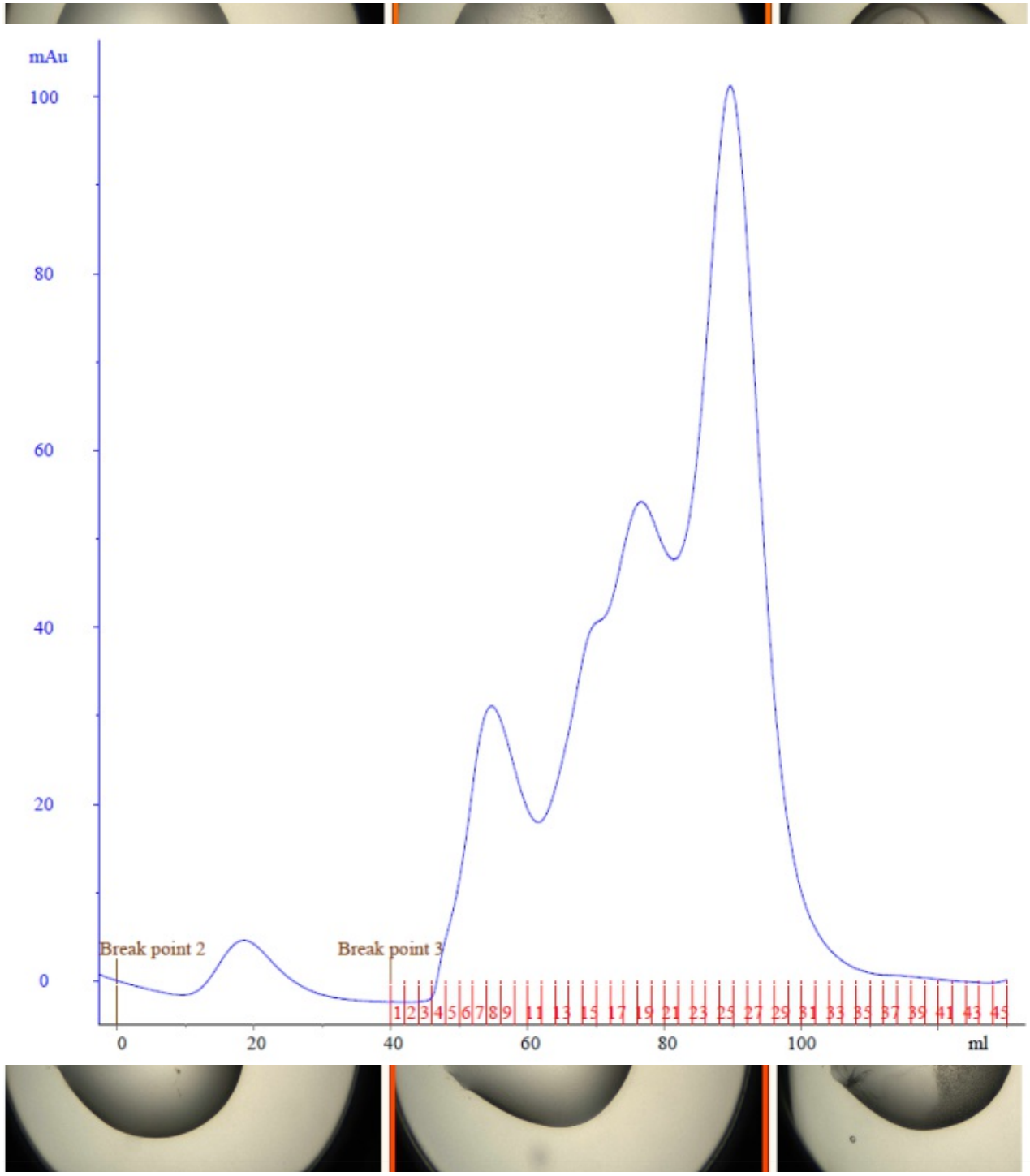

Condition: 0.2M ammonium acetate -- 25% PEG3350 -- 0.1M tris pH 8.5
Plate Stats
Projects: D68EV3CPR0A
Sort by last inspection date


Condition: 25% PEG3350 -- 0.1M tris pH 8.5
Plate Stats
Projects: D68EV3CPR0A
Sort by last inspection date

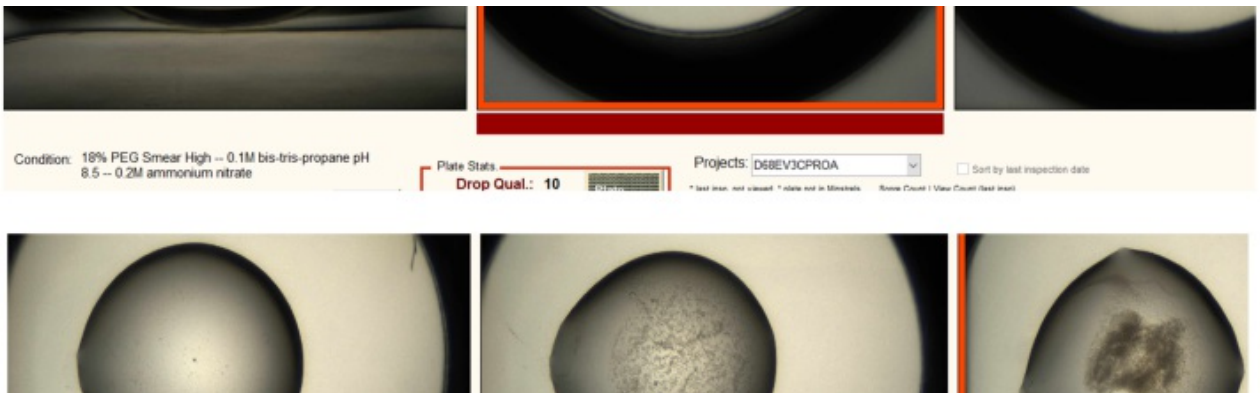

Condition: 0.2M trimethylamine N-oxide -- 20% PEG2000MME -- 0.1M tris pH 8.5
Plate Stats
Drop Qual.: 10
Projects: D68EV3CPR0A
Sort by last inspection date

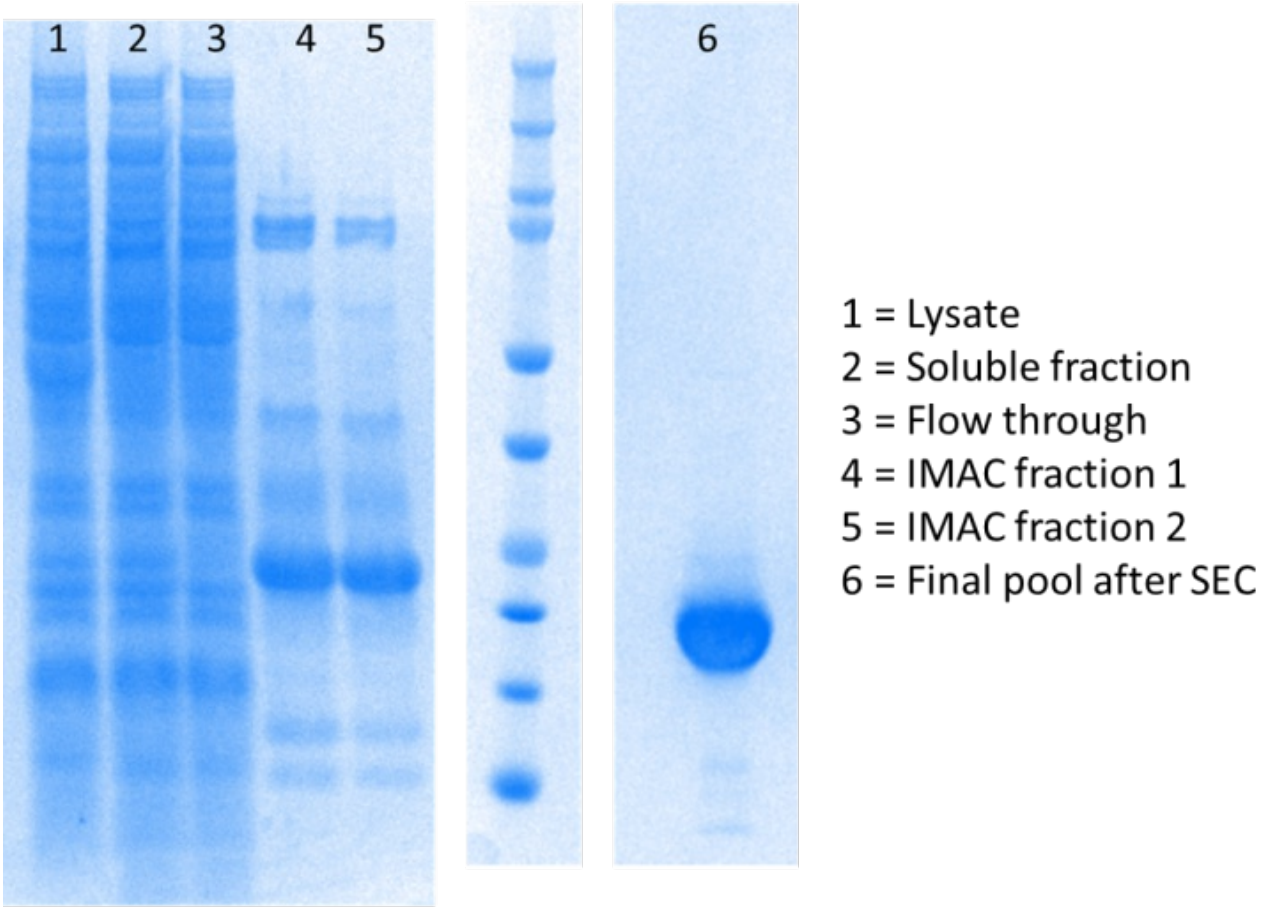


EV71A 3CL Protease superose 12 pg column

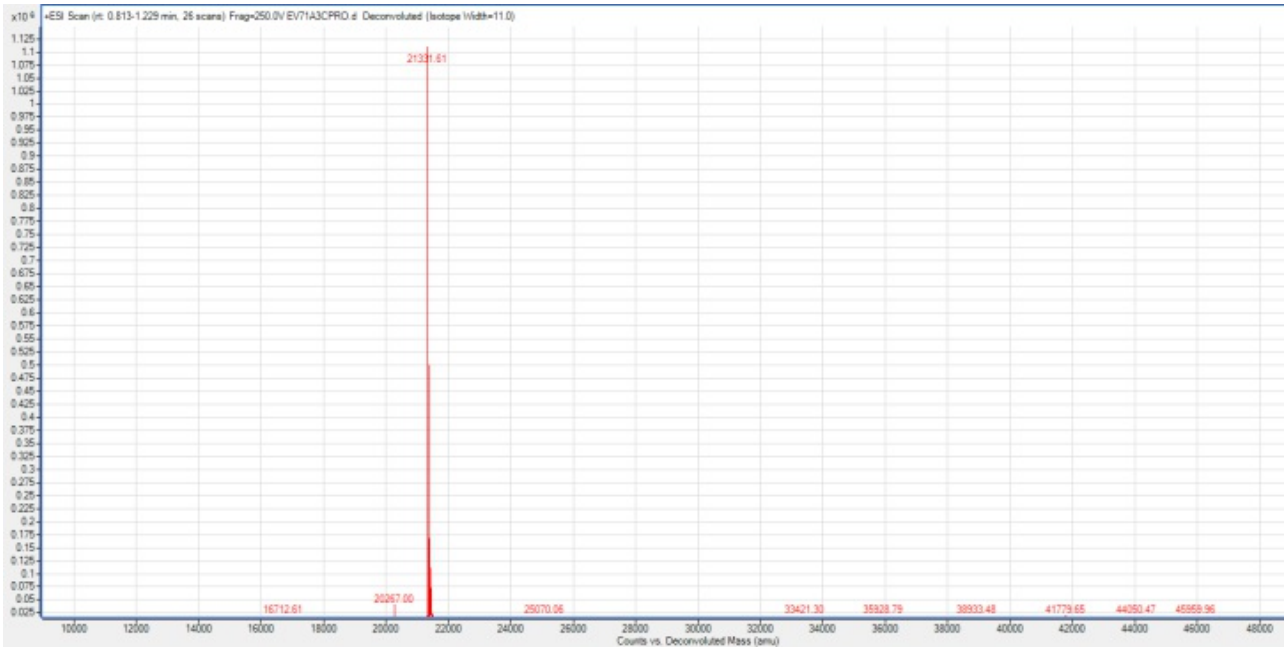


SDS PAGE A71EV3CPROA





A71EV3CPROA-p001 MS confirmation Z:\Agilent_SGC_QTOF\Rod\221124_MS424C



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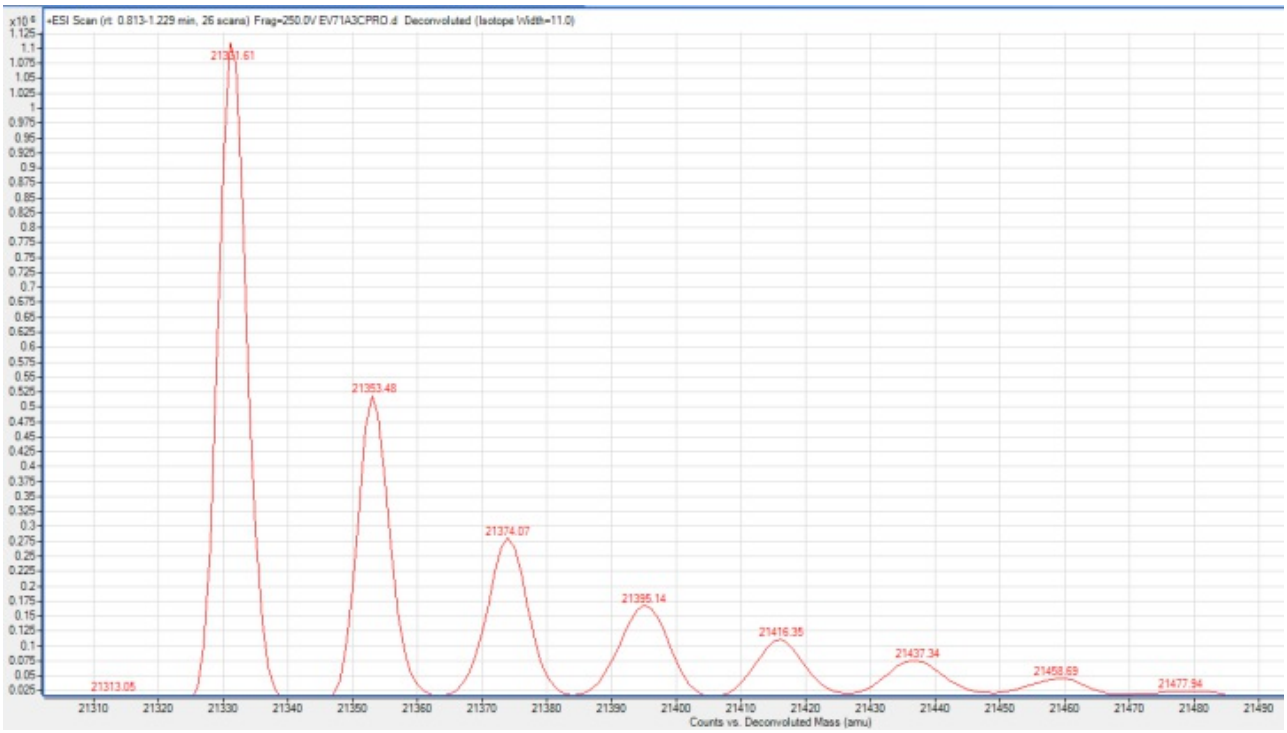


EVD71A 3CL Protease

Took peak centered around 90 mL and concentrated as much as possible did a total of 4 runs
Final yield after much concentrating is 2mL at A280 of 3.4 (341 uM) 7.3 mg/mL

Mass-Spec shows MW 21331.61 Da observed versus 21331.5 Da expected

A71EV3CPROA-p001
0.34 mM 55 x 50 uL aliquots after making plates
300 nL drops in

CI084990 LFS6
CI084991 BCS
CI084992 MORPHEUS
CI084993 HIN3
CI084994 JCSG7
CI084995 HCS3

Difficulty in cocentrating to 1mM in base buffer so I took a 50 uL aliquot and added either
0.5 mL of 50 mM Bicine pH 8.5, 500 mM NaCl, 5 % Glycerol, 0.5 mM TCEP
or
0.5 mL of 50 mM MES pH 6.5, 500 mM NaCl, 5 % Glycerol, 0.5 mM TCEP

Added to 0.5 mL 10000 MWCO cocentrator and reconcentrated

MES one would not go beyond original concentration of 0.34 mM
Bicine concentrated nicely to 1 mM

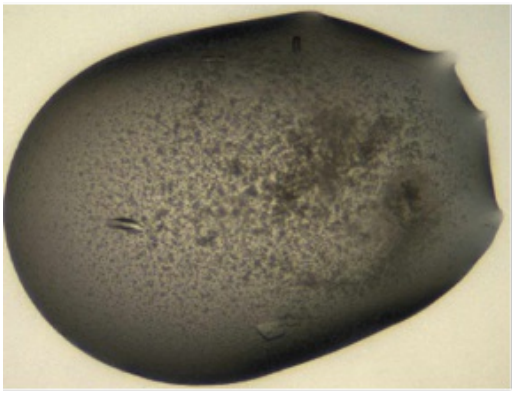
Thawed several more aliquots and repeated at scale to get enough to repeat original coarse screen plates

Buffer of protein now 10 mM Bicine pH 8.5, 500 mM NaCl, 5 % Glycerol, 0.5 mM TCEP and protein at 1 mM (21.3 mg/mL)

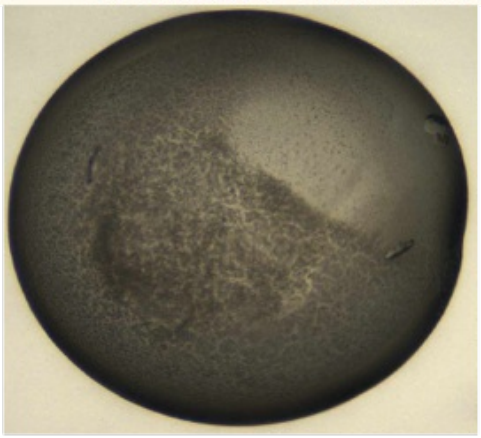
CI084900 BCS
CI084901 LFS6
CI084902 HIN3
CI084903 JCSG
CI084904 HCS3

After 4 days clear xtals

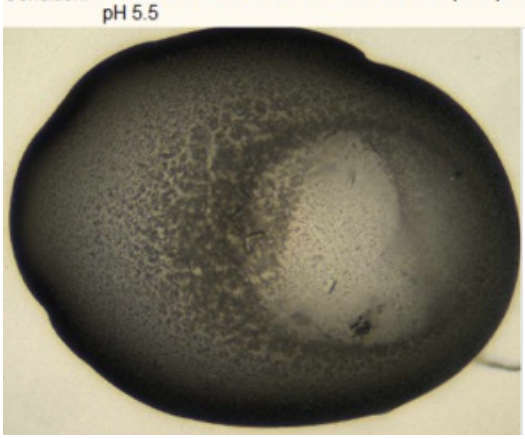
Condition: 30% PEG Smear Low -- 0.1M citrate/phosphate pH 5.5



Condition: 22.5% PEG Smear Medium -- 0.1M PIPES pH 7.0 -- 0.1M calcium chloride -- 0.1M magnesium chloride



Condition: 25% PEG Smear Medium -- 0.1M citrate/phosphate pH 5.5



Condition: 30% PEG4000 -- 0.2M magnesium chloride -- 0.1M tris pH 8.5