AVIDD ASAP: A71EV2A trunc08 A71EV2A-c012 Expression and Purification

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Date Started: **2023-Feb-02**
Experiment Started:
Projects: **Expression**;**Purification**;**ASAP**
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A71EV2A-c012/A71EV2A-k003/A71EV2A-e003/A71EV2A-p003

A71EV2A _F_trunc08 A71EV2A-c012

MHHHHHHGSGDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMTTHLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGM
EEEDVIEVYQEQTGGSGAIYVGNYRVVNRHLATHNDWANLVWEDSSRDLLVSSTTAQGCDTIARCDCQTGVYYCSSRRKHYPVSFSKPSL
IFVEASEYYPARYQSHLMLAVGHSEPGDCGGILRCQHGVVGIVSTGGNGLVGFADVRDLLWLDEEAMEQ—

Number of amino acids: 249

Molecular weight: 27927.21

Theoretical pI: 5.81

Cut
SUMO tag

MHHHHHHGSGDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMTTHLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGM
EEEDVIEVYQEQTGG
Number of amino acids: 105

Molecular weight: 12069.48

Theoretical pI: 5.96

A71EV2A-c012

SGAIYVGNYRVVNRHLATHNDWANLVWEDSSRDLLVSSTTAQGCDTIARCDCQTGVYYCSSRRKHYPVSFSKPSLIFVEASEYYPARYQS
HLMLAVGHSEPGDCGGILRCQHGVVGIVSTGGNGLVGFADVRDLLWLDEEAMEQ—

Number of amino acids: 144

Molecular weight: 15875.74

Theoretical pI: 5.58

Extinction coefficients:

Extinction coefficients are in units of M^{-1} cm^{-1}, at 280 nm measured in water.

Ext. coefficient 28795
Abs 0.1% (=1 g/l) 1.814, assuming all pairs of Cys residues form cystines

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Expression

Transformed BL21[DE3]RR with **A71EV2A-c012** (/kanR)

Grew 100 mL o/n in SOC + Kan

Used 10 mL to inoculate 1 L FORM-TB + Kan (6 L total)

Grew 4h 37C 180 rpm shaking (OD600 = 1)

Grew 18C 180 rpm shaking 1h

Added 0.5 mM IPTG final conc.

Grew o/n 18C 180 rpm shaking

Harvested 4000g 12C 20 minutes

Froze pellet -80C (final wcw = 100 g/L 17 g total)

Formedium TB custom

12 g/L tryptone

24 g/L yeast extract

3.3 g/L Ammonium Sulphate (NH4)2SO4

6.8g /L Potassium Dihydrogen Phosphate KH2PO4

7.1 g/L DiSodium Hydrogen Phosphate Na2HPO4 7.1

0.15 g/L Magnesium Sulphate MgSO4

0.03 g/L Trace Elements

55.85 g/L in MilliQ water added 20 mL 50 % glycerol

Autoclave

Add 1 mL 10% Antifoam 204 and Antibiotic

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Purification

Dissolve pellet in 4 mL per g using Lysis buffer (10 mM HEPES pH 7.5, 500 mM NaCl, 5 % glycerol, 0.5 mM TCEP, 1 % TX-100, 30 mM Imidazole, 0.5 mg/mL lysozyme, 0.01 mg/mL benzonase)

After dissolveing (1h RT stirring) incubate on ice 1h and then centrifuge 30000 g 1h 4C

Pass over 20 mL Ni-Sepharose-FF

Wash 3 x 100 mL Wash buffer (10 mM HEPES pH 7.5, 500 mM NaCl, 5 % glycerol, 0.5 mM TCEP, 30 mM Imidazole)

Elute 3 x 25 mL Elution Buffer (10 mM HEPES pH 7.5, 500 mM NaCl, 5 % glycerol, 0.5 mM TCEP, 500 mM Imidazole)

Pool peak fractions

50 mL at A280 of 12.7

Add 1:100 sumo protease (Senp1) and dialyse o/n against 1 L Wash Buffer

Pass over 20 mL Ni-Sepharose-FF (collect flow through)

Wash with 2 x 30 mL Wash Buffer 30 mM (collect flow through)

Wash with 2 x 30 mL Wash Buffer 60 mM (collect flow through)

Pool peak fractions

portein comes off predominately at 60 mM Imidazole but a lot of SUMO still prsent

Pass over 20 mL Ni-Sepharose-FF (collect flow through)

Wash with 2 x 30 mL Wash Buffer 60 mM (collect flow through)

Calculate yield and check on gel still some SUMO (similar MW and pI)

Concentrate to 9 mg/mL using 10,000 MWCO concentrator, struggles to get above A280 of 20 so lets not push it

Yield at this stage is approx 8 mL with A280 of 16, about 72 mg

Do SEC using 5 mL aliquots (approx. 40 mg) on 125 mL superose 12 pg using 10 mM HEPES pH 7.5, 500 mM NaCl, 5 % glycerol, 0.5 mM TCEP as mobile phase

Concentrate peak fractions to 0.79 mM (12.5 mg/mL, A280 of 22.5)

Do MS to confirm MW and flash freeze in LN2 and store in 100 µL single use aliquots (2.4 mL 30 mg total)

MS looks as expected very small signal for SUMO2 tag

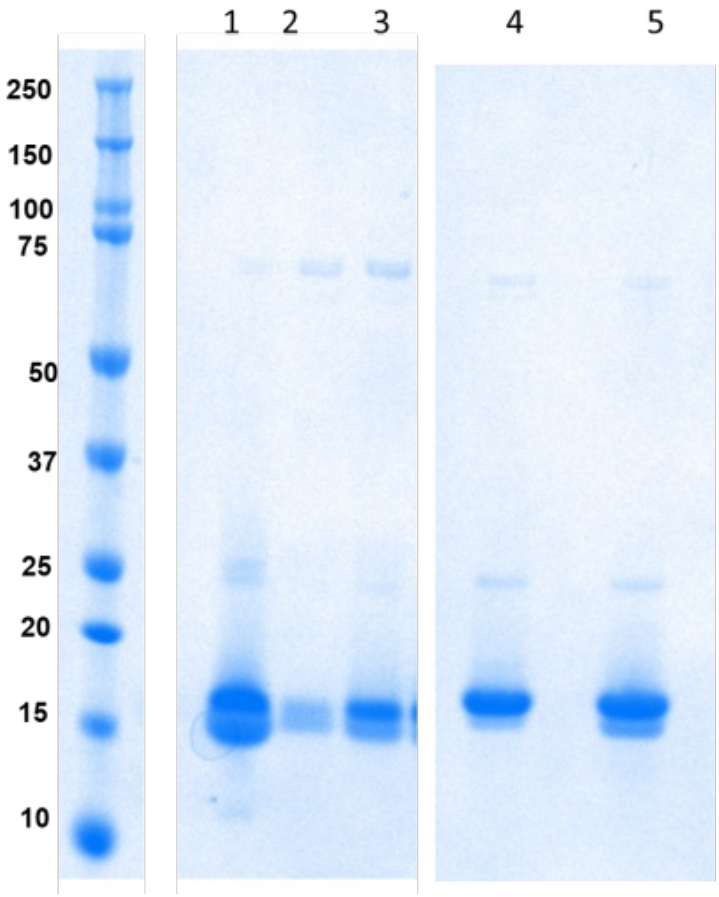
A71EV2A-c012

Expected = 15875.74
Observed = 15876.12

SUMO-tag

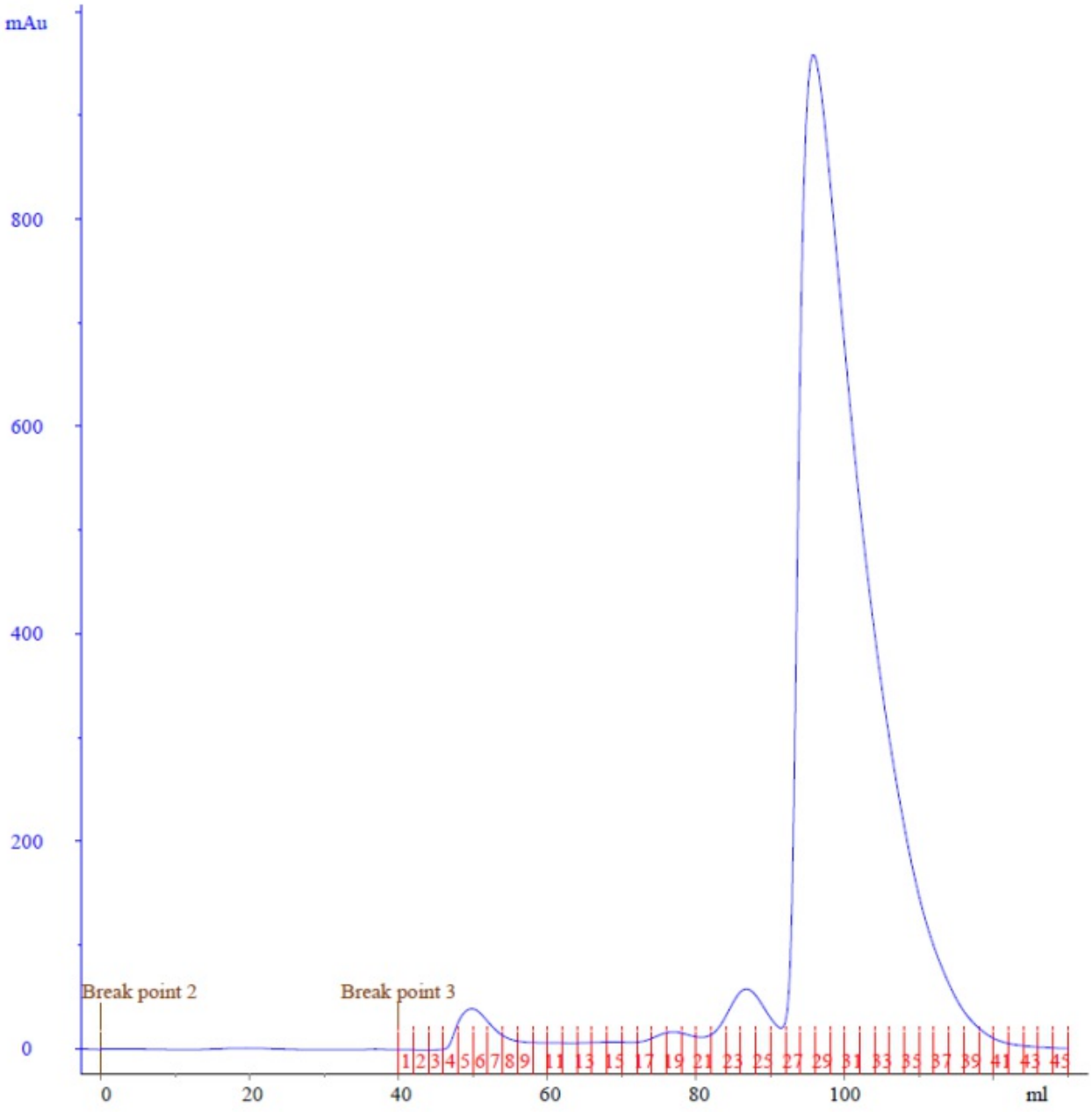
Expected = 12069.48
Observed = 12069 but 6.8x10^2 compared to 0.9 x10^7 for EVA712A

SDS-PAGE IMAC/revIMAC

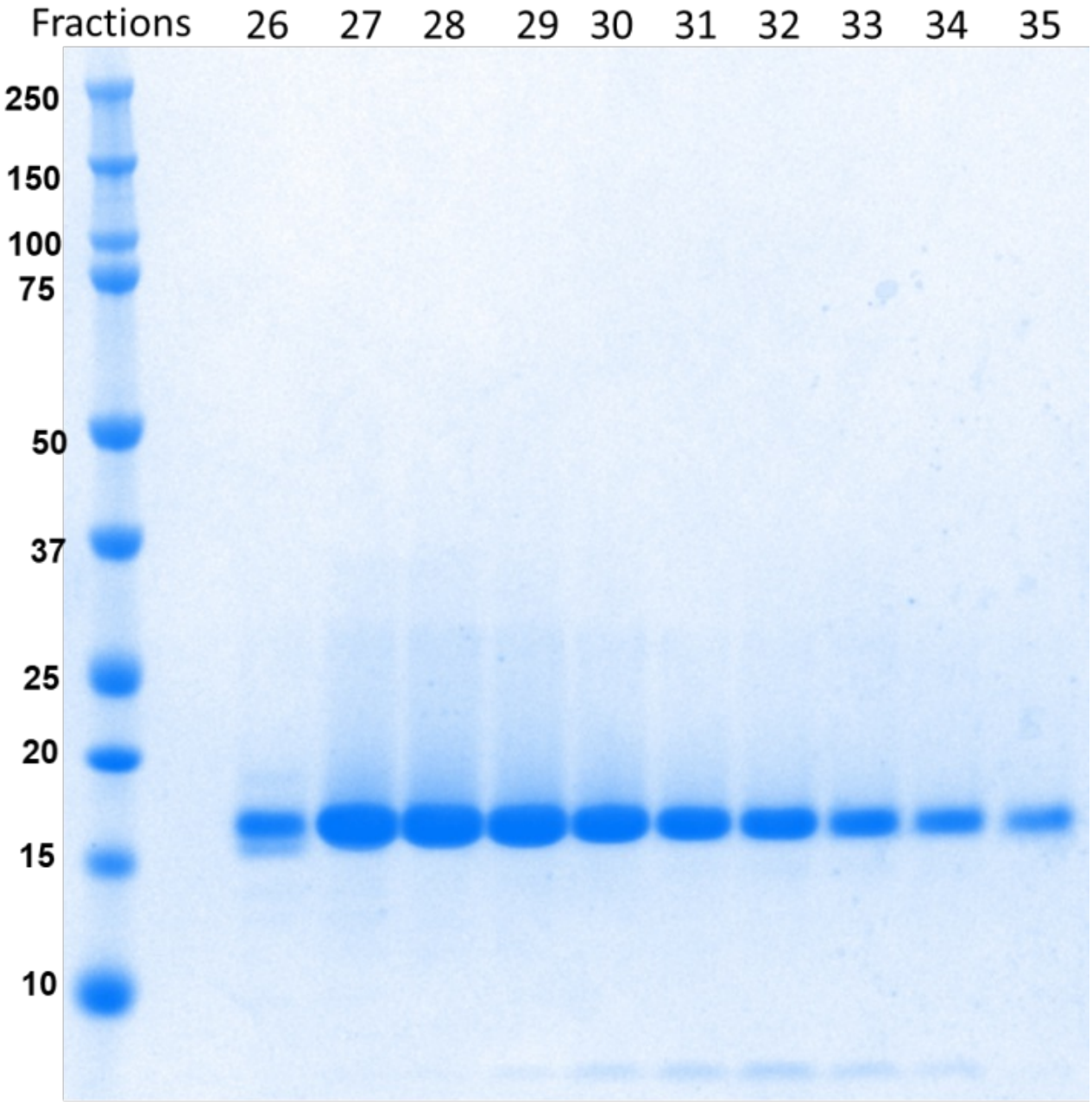


SUMO cleavage (SENP1)
1 = A71EV2A-c012, 27927.21 Da
2 = rev IMAC
3 = rev IMAC
4 = rev IMAC repeat
5 = rev IMAC repeat

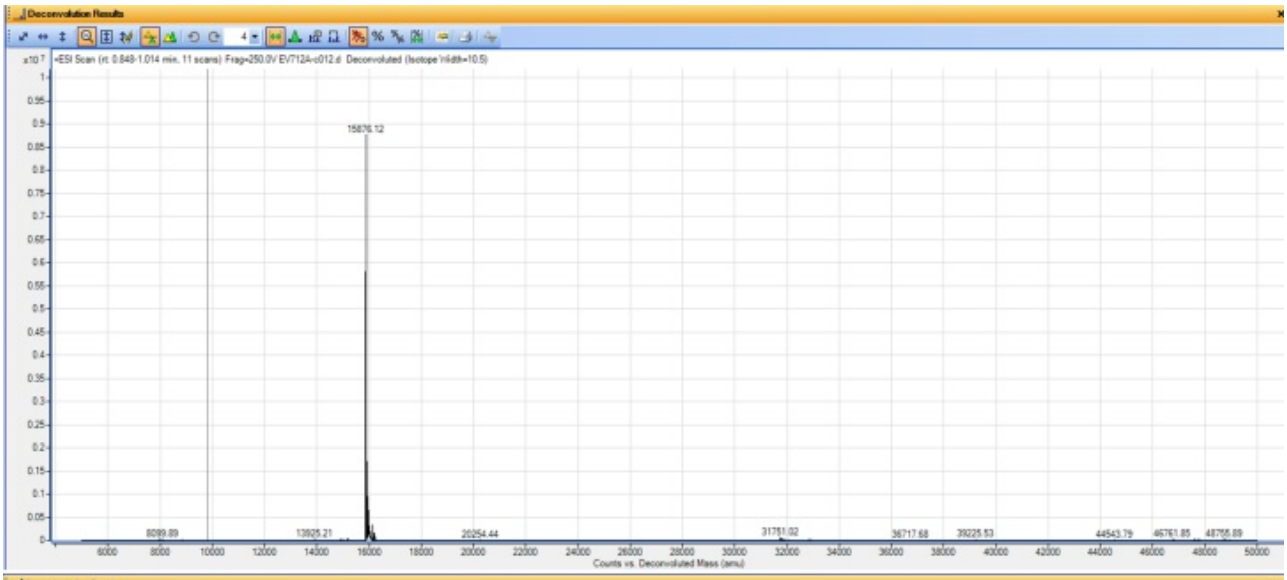
EVA712Ac012_125mL_SUPEROSE_12_PG_SEC_PROFILE_Large_Scale.pdf

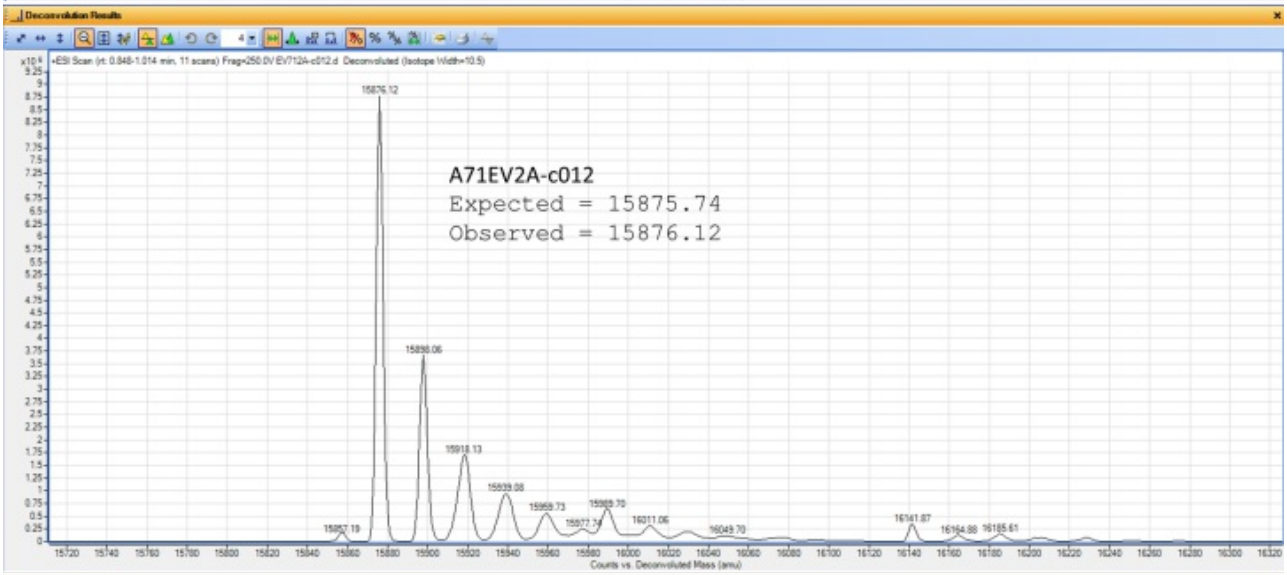


SDS-PAGE SEC FRACTIONS



Z:\Agilent_SGC_QTOF\Rod\230210_MS424H


Deconvolution Results
15876.12
Counts vs. Deconvoluted Mass (amu)


Deconvolution Results
15876.12
15838.06
15918.13
15839.08
15958.73
15957.19
16011.06
16049.70
16161.87
16164.88
16185.61
A71EV2A-c012
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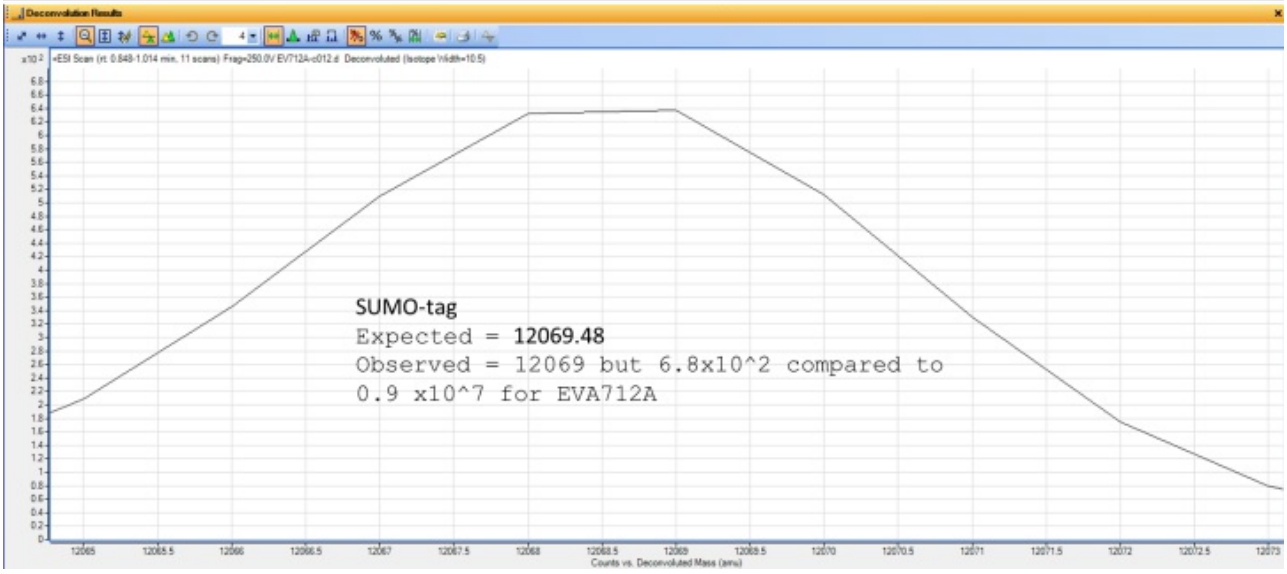
Observed = 15876.12
Counts vs. Deconvoluted Mass (amu)


Deconvolution Results
SUMO-tag
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Observed = 12069 but 6.8x10^2 compared to
0.9 x10^7 for EVA712A
Counts vs. Deconvoluted Mass (amu)