

2L Purification of JMJD1AA-c223 [JMJD1AA-p043]

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Projects: Epigenetics Project;Purification

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JMJD1AA-c223	UO-129-baculo	B12	SF9	pFB-CT10HF-LIC	JMJD1AA-k817	JMJD1AA-e413	JMJD1AA-p043

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IMAC

lysis/wash10: 50mM HEPES-KOH, 0.3M kCl, 5% glycerol, 10mM imidazole, Ph 7.5

wash40: 50mM HEPES, 0.3M kCl, 5% glycerol, 40mM imidazole,

elution: 50mM HEPES, 0.3M kCl, 5% glycerol, 250mM imidazole,

GF

20mM HEPES pH 7.4, 0.3M kCl, 5% glycerol, Ph 7.5

Expression 1/3/13

Cells were infected with 3ml of virus at around 2×10^{10} density (done by S.Goubin). Cells were coming from batch cultivated in sf900 media and then passaged twice in isenct express. This is important since protein from this prep is really stable what couldnt be observed in previous purifications. **So if you won't be able to repeat it I would reccomend to do the expression in sf90 medium.**

4/3/2013

2L of cells were harvested on monday by centrifugation at 900xg for 15min-no wash in PBS- lots of protein is lost during that step.

Cells were resuspended in IMAC 35ml of lysis buffer + inhibitors set VII+inhibitors set III and (1:500 each).

Lysate was homogenized in douce homogenizer - 30 strokes, then clarified by centrifugation for 45min at 21krpm.

To the supernatant 1ml of equilibrated Sepharose 6 FF resin was added and proteins were bound for 45min. Resin was spun down 500xg 15min and transferred into gravity column. Resin was washed with 20CV of binding buffer and 10CV of wash40 buffer. Eluted with elution buffer 5x1ml (flow was stopped after each elution).

Elution fractions 1-5 were pooled, filtrated and loaded onto Superdex200 column. Flow 1.2ml/min

Last peak was pooled and concentrated to 1.5mg/ml on amicon 50MWCO.

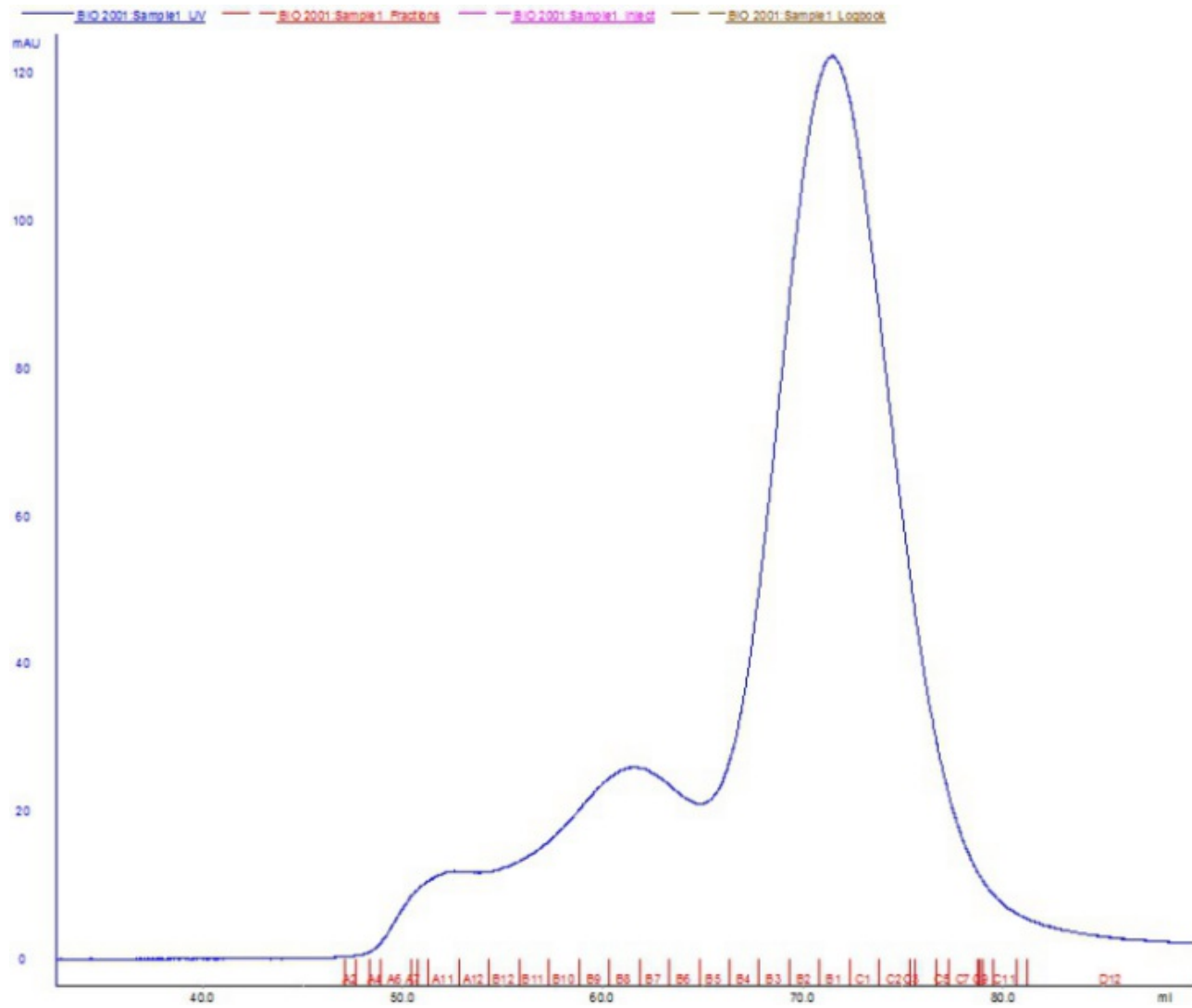
p43

Total: 2.7mg

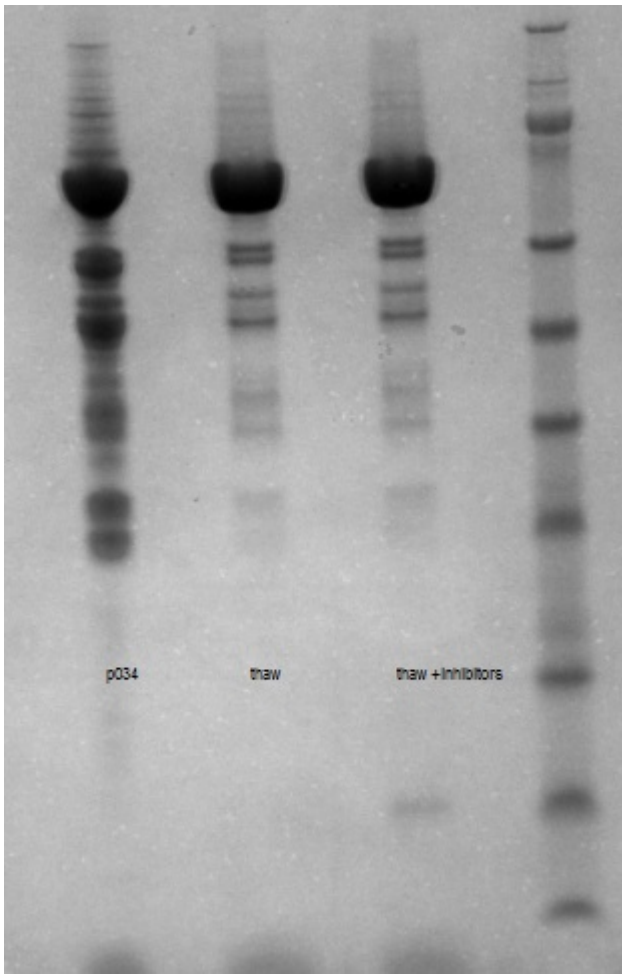
concentration: 1.5mg/ml
aliquots: 61x 30ul

Protein active

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p034 and p043 after freezing and thawing



FDH: blue-positive control red-negative

Signal Curve(s)

