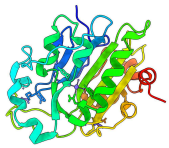
Feb 19, 2026 Version 2

Purification of SerMNucA (Benzonase/Turbonuclease) as 10xHis, MBP or myc-tag fusions V.2



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We use this protocol and it's working

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Abstract

From the Author (KL) : all the plasmids are available here > > https://www.addgene.org/browse/article/28244500/

During the lysis of cells, in particular of eukaryotic origin, there is a large release of nucleic acids. The presence of DNA/RNA increases the viscosity of a lysate and may pose problems during downstream processes. Removal of nucleic acids can be performed in many ways, of which one is by enzymatic digestion by nucleases. DNase/RNase are commonly added during protein purification alongside other commercial enzymes such as Benzonase (Novagen) and Turbonuclease. The latter enzymes are industrially produced or recombinant versions of the Serratia marcescens nuclease. This enzyme, hereafter referred to as SerMNucA, is able to hydrolyze both single- and double-stranded DNA and RNA with high activity.

Reported methods have shown that the nuclease can be made as inclusion bodies followed by refolding or secreted in the extracellular media. Here we report the purification of the SerMNucA nuclease in a soluble form, followed by purification from either periplasm or cell lysate. We find that purified protein from either fraction are active in a salmon sperm degradation assay with the periplasmic fraction slightly more. One can choose to purify from either fraction or from the total lysate.

This protocol allows for a lab to independently produce tagged and untagged versions of the SerMNucA endonuclease in large quantities that may be required for certain experiments that may be cost prohibitive otherwise.

3 versions are reported here:

10x-His tagged
MBP tagged (From SGC Toronto/Oxford)
myc tagged

The use of the enzyme is in such small quantities that repurification with the tag should not be an issue. The MBP tagged version is cleavable with TEV protease (see also: **10x-His-SuperTEV**). Finally the myc-tagged version should not interfere with any common protein purification protocols.

References:

Benedik and Strych. (1998) FEMS Microbiology Letters
Biedermann et al. (1989) Carlsberg Research Communications
Friedholl et al. (1996) Nucleic Acids Research

Guidelines

This protocol is written with the expectation of standard bacterial culture and basic purification knowledge

Materials

Plasmid #1
Plasmid #2
Plasmid #3

BL21 (DE3) cells (Lucigen)
TB + trace elements (Formedium)
Glycerol (Applichem)
Akta system (Cytiva)
HisPur NiNTA resin (Pierce/Thermo)
Amylose resin (NEB)
Hitrap Q HP 5 mL column (Cytiva)
12-14 kDa dialysis tubing (SpectraPor)
Dialysis clips (SpectraPor)
Sonicator
5 M NaCl
1 M HEPES, pH 7.5
1 M TRIS, pH 8.0
0.5 M EDTA, pH 8.0
2.5 M imidazole, pH 7.5 (We recommend, Sigma #56749-1KG for low background absorbance)
1 M MgCl2
1 M IPTG
Sucrose
4X LDS loading dye (Genscript)
NuPage 4-20% SDS-PAGE Gels (Thermofisher)
8M Urea
Salmon testes DNA (Rockland)
Plastic disposable or glass reusable columns (Biorad)

Before start

Recommended to have ready before starting alongside Materials.

-Autoclaved flasks and bottles
-Autoclaved LB media
-Autoclaved TB media
-Buffers
-Liquid nitrogen

Growing bacterial cultures (2L) and periplasmic extraction

4d

1 Transform the bacterial plasmid expressing the construct of interest (His, MBP or myc fusions) into BL21 (DE3) cells. Plate on to LB-Agar plates + Kanamycin. Grow Overnight]at Temperature 37 °C or over the weekend at Temperature 25 °C .

1d

2 In an afternoon, pick a streak of cells and innoculate into 200 mL of LB + Kanamycin media. Note: for every 1 L of expression culture, you will require 10-20 mL of pre-culture. Grow Overnight at 37 °C

1d

3 The next morning, add MgCl2 to [M] 2 millimolar (mM) , innoculate 20-40 mL of preculture into 1 flasks containing 2 L of TB media (2 L in a 5 L flask is appropriate). Shake in an incubator at 37 °C for 3-4 hours until OD600 ~0.8-1. Take a 1 mL sample of the culture, this will be used as a pre-induction control sample. Immediately change the temperature to 18 °C , add IPTG to [M] 0.5 millimolar (mM) , continue shaking overnight (approximately 20:00:00)

20h

4 Take a 1 mL sample of the culture and measure the OD600, this will be used as a whole cell post-induction sample. Harvest the culture by centrifuging at 5000 x g, 10°C, 00:30:00 .

30m

4.1 SerMNucA has been designed to be secreted into the oxidizing environment of the periplasm of E.coli to enable proper folding. Proteins from this particular compartment can be extracted by osmotic shock.

10m

Prepare the following:

	A	B	C
	Buffer	Component	Concentration (mM)
	TES 1X	TRIS, pH 8	200
		EDTA, pH 8	0.5

	A	B	C
		Sucrose	500

5 For every 2 L of culture, add 20 mL of ice-cold 1X TES buffer and gently rotate or mix for at least 02:00:00 at 4 °C. This will resuspend the pellet without disrupting the cell membrane or cell wall. 5h

Then mix 30 mL of ice-cold 0.25 X TES buffer (ie. 1X TES diluted with water to 0.25X) to the resuspend pellet above to generate the osmotic shock. This mixture is then rotated and mixed for another 02:00:00 at 4 °C. The periplasmic shocked mixture was recovered by centrifuging at 20000 x g, 10°C, 00:30:00. The supernatant contains the periplasmic extract and desired protein. Take a 1 mL sample. The pellet can be saved if one choose to purify from this compartment as well. Both fractions can be stored at -20 °C.

6 Confirm expression by running an SDS-PAGE gel. 1h

Recommended recipe to prepare SDS-PAGE samples. Mix 1:4, 4X LDS loading dye and 8 M Urea, to make a Urea loading dye.

For samples before and after induction with IPTG, calculate the amount of sample needed to prepare. We use the following formula:

1/OD600 × 100 uL = **volume in uL to centrifuge down** 14000 rpm, 00:01:00.

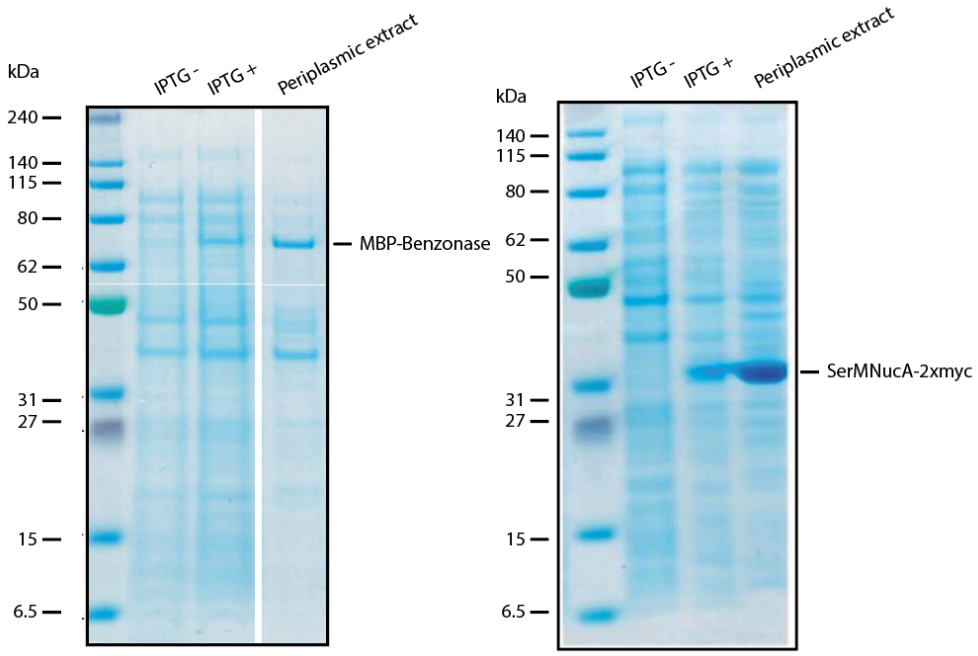
Discard the supernatant, Keep the pellet.

Resuspend pellet in 40 uL of the Urea loading dye.

For the osmotic shock supernatant, mix 30 uL of sample and 10 uL of 4X loading dye

Load 10 uL on to a NuPage 4-12% bis-Tris SDS-PAGE gel.

Expected result



Typical expression pattern and periplasmic extracts are shown here

Prepare purification buffers

7 From stock solutions prepare the following as needed :
Filter 0.22 or 0.45 um

	A	B	C	D	E
	Buffer	*Component*	*Concentration (mM)*		

	A	B	C	D	E
	Wash buffer (1 L)	NaCl	700		
		HEPES, pH 7.5	20		
	Dialysis buffer	NaCl	100		
		TRIS, pH 8	100		
		MgCl2	10		
	If performing Ni-NTA				
	Ni-NTA Elution buffer (100 mL)	NaCl	700		
		HEPES, pH 7.5	20		
		Imidazole, pH 7.5	500		
	If performing Amylose				
	Amylose Elution buffer (100 mL)	NaCl	700		
		HEPES, pH 7.5	20		
		Maltose	10		
	If performing anion-exchange				
	Q-Low buffer (1 L)	HEPES, pH 7.5	20		
	Q-High (1 L)	NaCl	1000		
		HEPES, pH 7.5	20		

Buffer lists for purification

Option 1 : Purification by Ni-NTA (10xHis construct)

2h 40m

8 Defrost the pellet, and resuspend in 40 mL of wash buffer and glycerol to 10% v/v. Cells are lysed by sonication and clarified by 20000 x g, 4°C, 00:30:00 . Filter the supernatant with a 0.45 um membrane and supplement with imidazole to [M] 25 millimolar (mM) .

Defrost the periplasmic extract and supplement with imidazole to [M] 25 millimolar (mM) .

40m

9 Add 5 mL of settled NiNTA resin already equilibrated in wash buffer to the supernatant (SN) and the periplasmic extract. Mix the resin gently for 02:00:00 at 4 °C

2h

10 Transfer the resin to a column and collect the flowthrough (FT). The resin is then washed and the target protein eluted as follows:

1h

	A	B	C
	Step	**Concentration imidazole (mM) / %Elution buffer**	**Volume (mL)**
	Wash 1	20 mM / 4%	4 × 25 mL
	Wash 2	50 mM / 10%	4 × 12.5 mL
	Wash 3	100 mM / 20%	2 × 12.5 mL
	Elute 1	300 mM / 60%	5 × 10 mL
	Elute 2	500 mM / 100%	1 × 15 mL

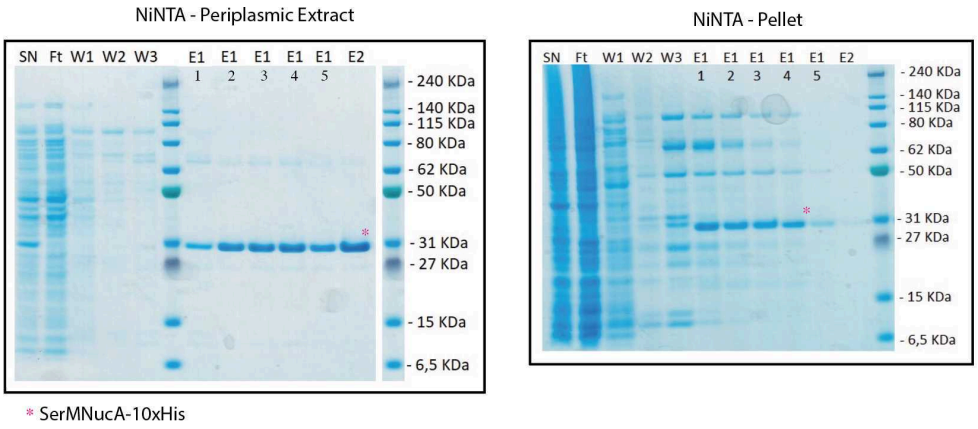
Wash and Elute buffers are made by mixing proportionally from the stock Wash and Elution buffers

11 Fractions from each step are run on a gel and the purest fractions are pooled. A typical purification is as follows.

40m

Fractions containing the target protein at a desired purity can be pooled and dialyzed into dialysis buffer overnight at 4 °C .

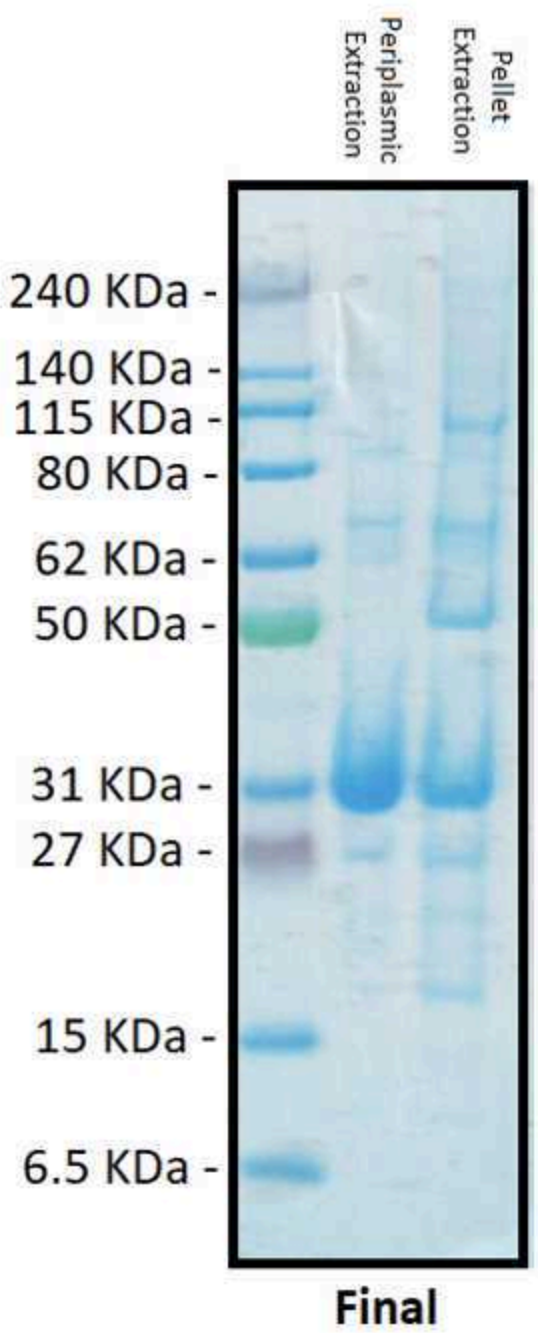
Expected result



Typical SDS-PAGE gel of a purification from both periplasmic extract and cell pellet. Additional bands purified from the cell pellet could be potential contaminants requiring additional purification steps or higher order oligomers.

12 After dialysis, the sample was concentrated to around [M] 1 mg/mL diluted with glycerol to [M] 50 % (v/v) and run on a gel to verify integrity and purity. The purified SerMNucA-10xHis can be stored at -20 °C . 30m

Expected result



Sample after dialysis and dilution with glycerol

Note

1) Increased purity can be achieved by adding additional steps. (See anion exchange section or by size-exclusion chromatography)

2) Both the periplasmic and cell pellet gives active protein (See activity assay section). However the purified protein from the cell pellet is observed to be forming higher-order oligomeric states. This is most likely mediated by improper disulfides formed within the cytosol. The periplasmic extract does not contain these species.

Option 2 : Purification by Amylose (MBP construct)

4h 10m

13 Defrost the pellet, and resuspend in 40 mL of wash buffer and glycerol to 10% v/v. Cells are lysed by sonication and clarified by 20000 x g, 4°C, 00:30:00 . Filter the supernatant with a 0.45 um membrane. 30m

Defrost the periplasmic extract.

14 Add 5 mL of settled amylose resin already equilibrated in wash buffer to the supernatant (SN) and the periplasmic extract. Mix the resin gently for 02:00:00 at 4 °C 2h

15 Transfer the resin to a column and collect the flowthrough (FT). The resin is then washed and the target protein eluted as follows: 1h

	A	B	C
	Step	%Elution buffer	Volume (mL)
	Wash	0	4 × 25 mL
	Elute	100	5 × 10 mL

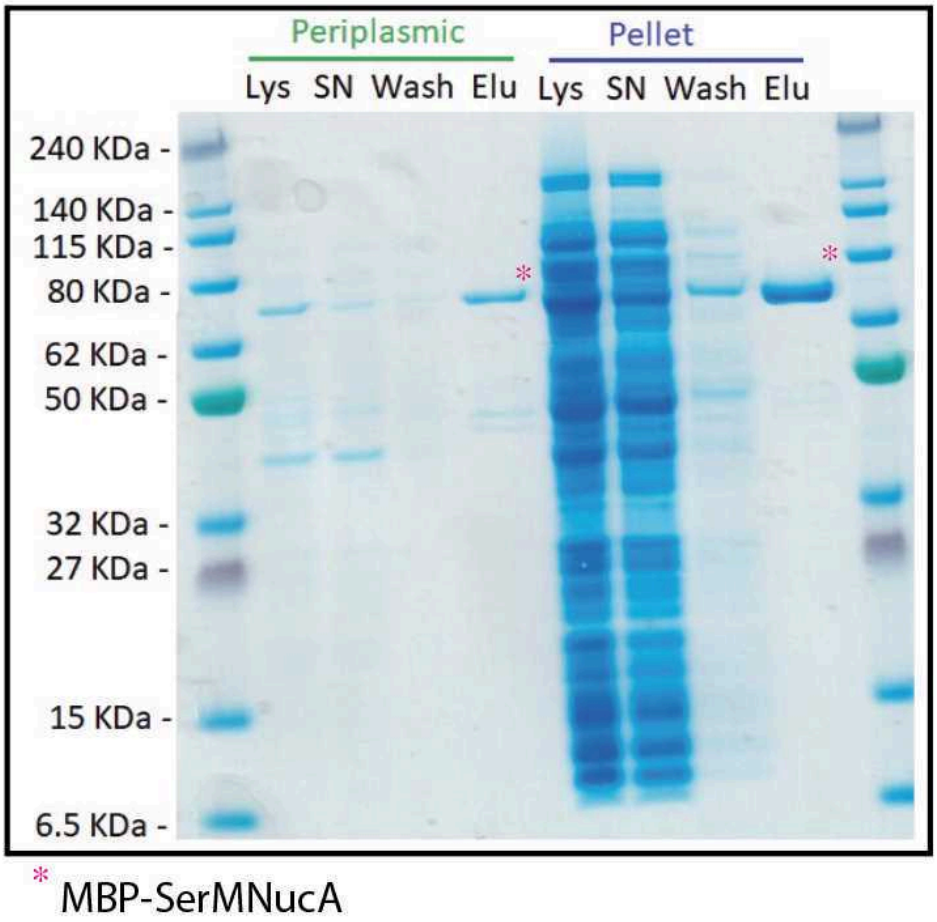
	A	B	C

Wash and Elute buffers are made by mixing proportionally from the stock Wash and Elution buffers

16 Fractions from each step are run on a gel and the purest fractions are pooled. A typical purification is as follows. 40m

Fractions containing the target protein at a desired purity can be pooled and dialyzed into dialysis buffer overnight at 4 °C .

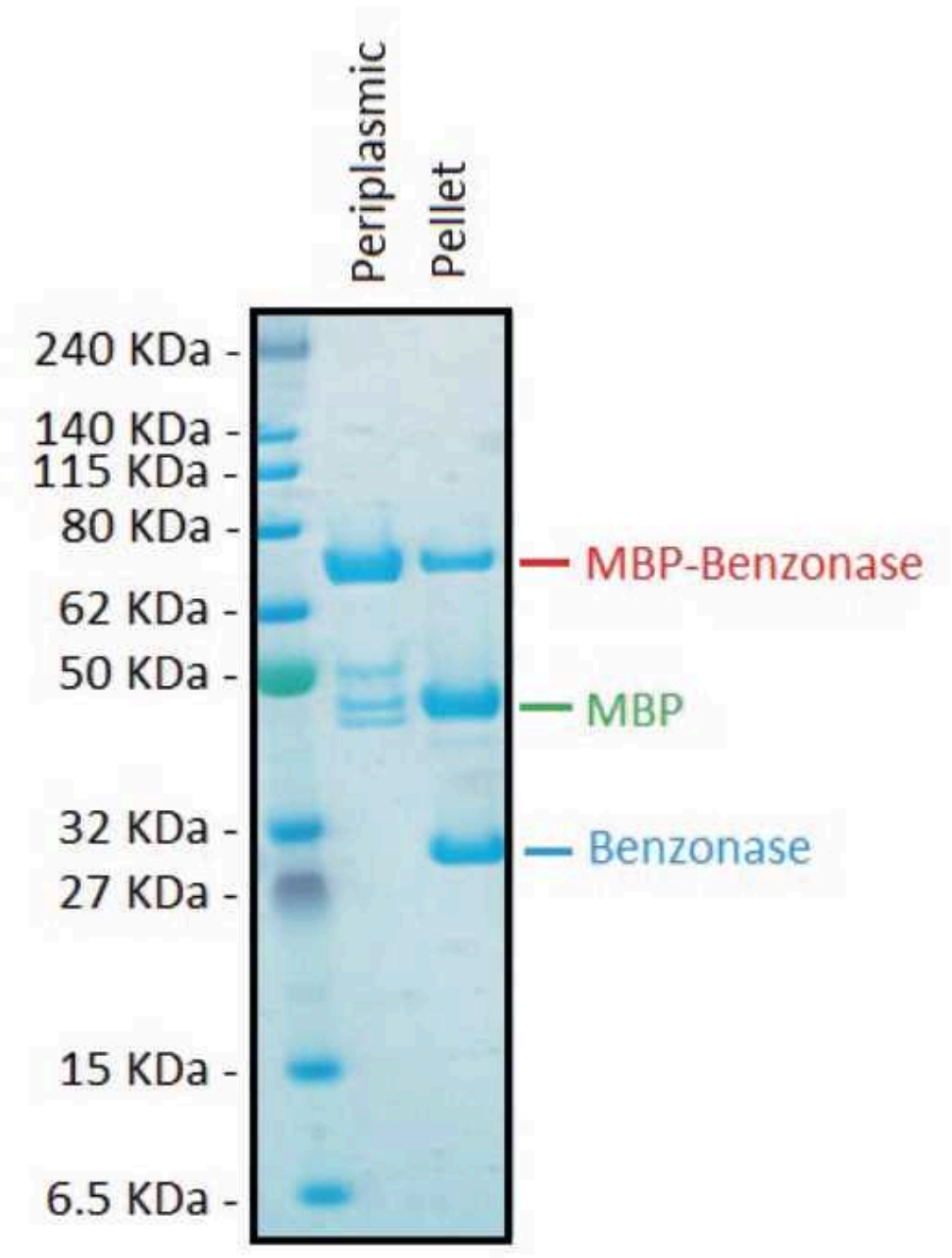
Expected result



Typical SDS-PAGE gel of a purification from both periplasmic extract and cell pellet.

17 After dialysis, the sample was concentrated to around [M] 1 mg/mL diluted with glycerol to [M] 50 % (v/v) and run on a gel to verify integrity and purity. The purified MBP-SerMNucA can be stored at -20 °C .

Expected result



Sample after dialysis and dilution with glycerol

Note

1) We observed degradation of the fusion construct. Increased purity can be achieved by adding additional steps. (See anion exchange section or by size-exclusion chromatography).

2) TEV cleavage by TEV protease can also be performed. It should be noted that one should remove the protease if downstream use are with protein samples that contain TEV or 3C protease cleavage sites.

3) Both the periplasmic and cell pellet gives active protein (See activity assay section).

Option 3 : Purification by Anion-Exchange Chromatography (myc tagged)

30m

18 Defrost the pellet, and resuspend in 40 mL of Q-Low buffer and glycerol to 10% v/v. Cells are lysed by sonication and clarified by 20000 x g, 4°C, 00:30:00 . Filter the supernatant with a 0.45 um membrane. 30m

or

Defrost the periplasmic extract.

19 Using an AKTA system, load the lysate sample or periplasmic extract on to a 5 mL HiTrap Q column (5 mL = 1 CV) equilibrated with Q-Low buffer.

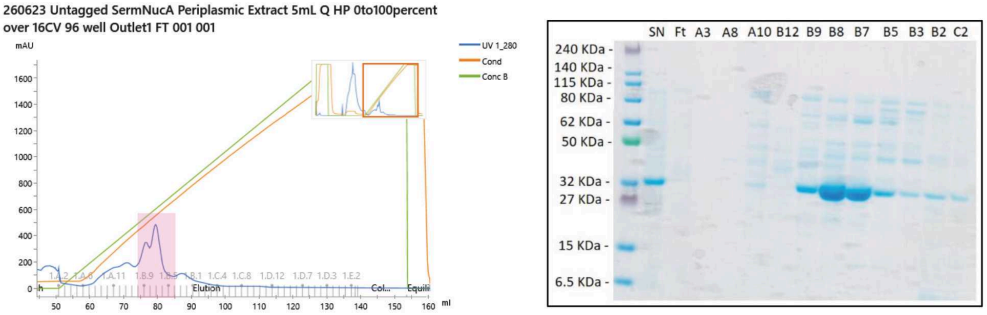
Once loaded, run a gradient from 0 to 100% Q-High buffer over 16 CV.

The protein should elute between ***20-30 mS/cm of conductivity***

Fractions from each step are run on a gel and the purest fractions are pooled. A typical purification is as follows.

Fractions containing the target protein at a desired purity can be pooled and dialyzed into dialysis buffer overnight at 4 °C .

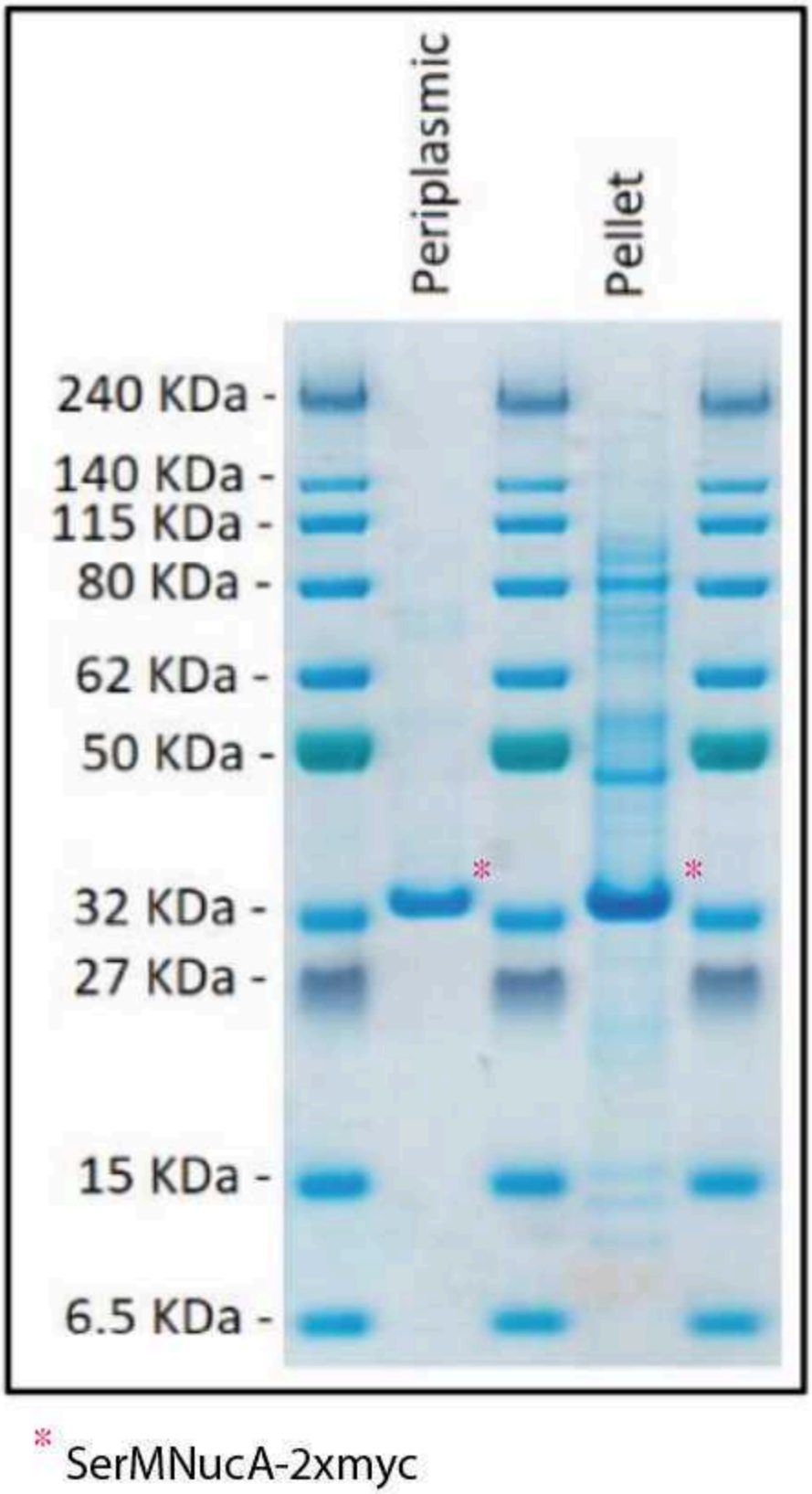
Expected result



Typical chromatograph and gel of fractions

20 After dialysis, the sample was concentrated to around [M] 1 mg/mL diluted with glycerol to [M] 50 % (v/v) and run on a gel to verify integrity and purity. The purified SerMNucA-2xmyc can be stored at -20 °C.

Expected result



Sample after dialysis and dilution with glycerol

Note

1) The purification from the pellet is not shown. What is shown in the final sample was an anion exchange step as for the periplasmic fraction, followed by a size exclusion on fractions that were enriched with the band of correct size.

2) Both the periplasmic and cell pellet gives active protein (See activity assay section).

Activity Assay

30m

21

30m

1) Prepare Assay buffer (10 mL) : [M] 50 millimolar (mM) TRIS, pH 8, [M] 1 millimolar (mM) MgCl2

2) Dilute Salmon testes DNA to [M] 2 mg/mL in Assay Buffer

3) Dilute enzyme to [M] 0.005 mg/mL in Assay Buffer

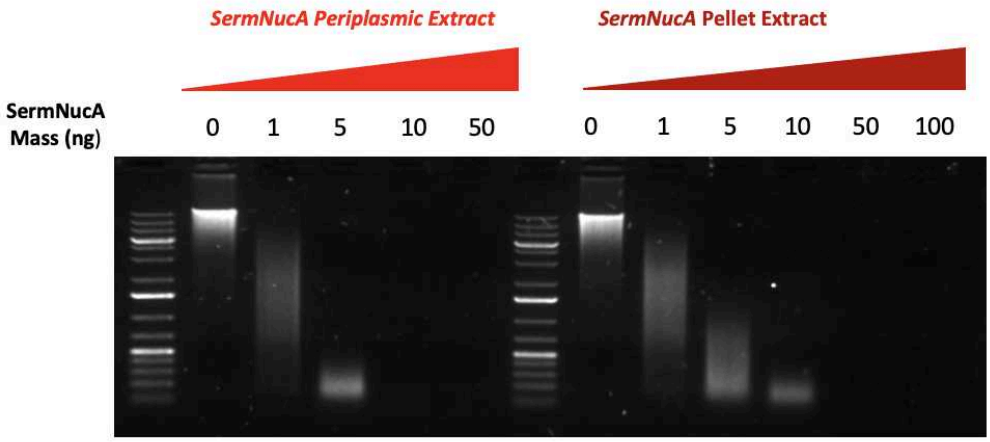
4) Each reaction contains 25.6 µL of Salmon Testes DNA + in increasing concentrations of enzyme, total volume was 50 µL of reaction completed with buffer. In this example, His-tagged nuclease purified from either the periplasm or the pellet fraction.

5) Incubate at 37 °C for 00:30:00

6) Visualize the DNA bands on a 1% Agarose gel

Expected result

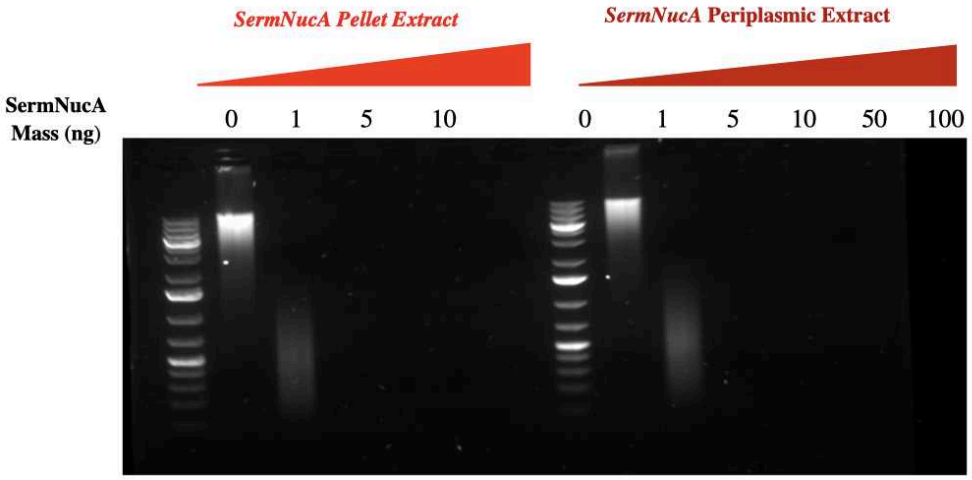
His tagged version



Expected result on an agarose gel showing the degradation to smaller DNA pieces in the presence of increasing concentrations of enzyme (His-tagged)

Expected result

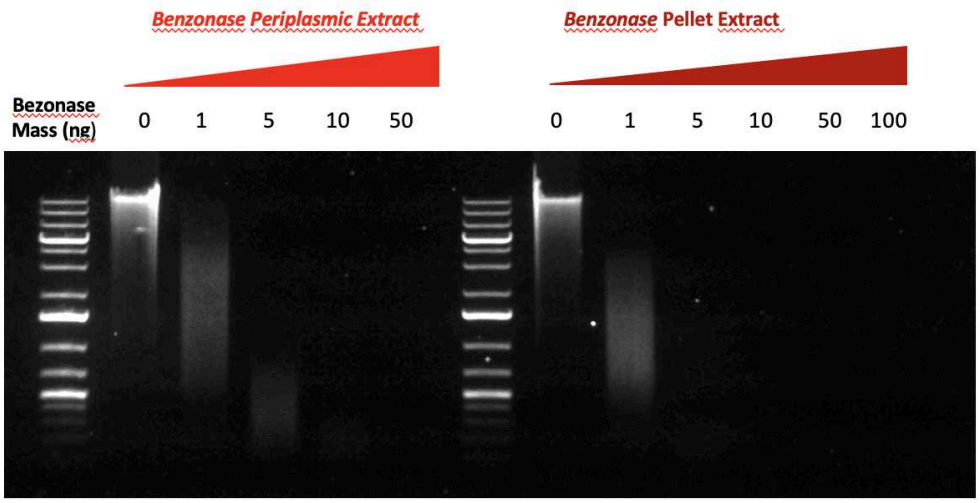
myc tagged version



Expected result on an agarose gel showing the degradation to smaller DNA pieces in the presence of increasing concentrations of enzyme (myc-tagged)

Expected result

MBP tagged version

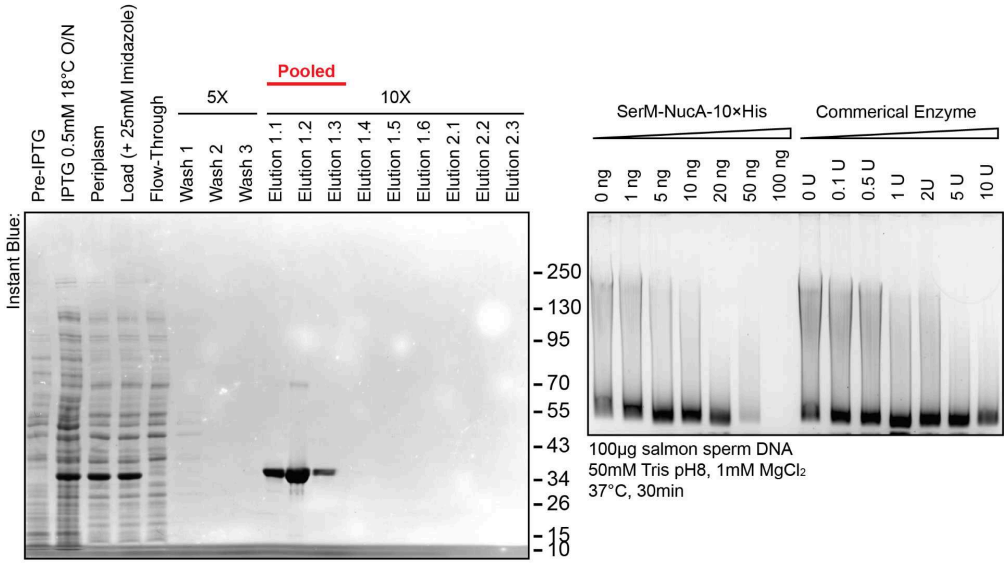


Expected result on an agarose gel showing the degradation to smaller DNA pieces in the presence of increasing concentrations of enzyme (MBP-tagged)

Additional Validation versus "Vendor" Nuclease

22 Data from Michael Lim (Kings College). His tagged protein was purified via gravity flow (no batch binding) and assayed with indicated conditions compared to a commercial vendor's enzyme using their indicated activity units

Expected result



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