

Expression & purification of 6his-SmPRMT3(1-553)

Construct

MGSSHHHHHHSSGTGSGENLYFQGHMSPCDQSSNYDLDMEPNDSSYFGSYGHFEIHGEMINDRVRTESYVNFILSN
AEKYFKHKIILDVGS GSGILSIIAAQAGASHVYGVEAADEIYAASHETLRVNNLLERVTFIHGQAESVELPVKKVDVIISE
WMGYFLFFESMLDSVLKMASKYLSRDGHIFPRHYTLNLLGVQCSEQLRKRRLHWNVYGYNMPALRRAALSEAHV
LNLTNEHVTPPISPITILTQSFELVALDLDMMHRNRINYNSNHCSLLCEQKFHLTIQPTTDINNNSSSSSYELDAIVGYFD
VRFDDADCKVEFSTSPPTPLTHWKQTLFLDKPIRVKPGDKISGIITIRRATTDNRGLEINLLIGETENSLEIKQTFDLIG

Length = 396 AA

Molecular weight = 44782.3 Da

pI 5.66

$\xi(\text{red}) 40340 \text{ L.M}^{-1}$

Expresion

Grow pnEAvH_SmPRMT3-184 BL21(DE3) transformants on an LB-Amp plate (1 plate/L culture), overnight 37°C. Resuspend cells in LB medium (5 mL/plate) and inoculate liquid **2X** LB medium + 100 $\mu\text{g/mL}^{-1}$ Ampicilline. Grow culture(s) at 37°C, 200 rpm and measure OD600 every 20 minutes. When OD600 reaches 0.4, set temperature to 20°C. When OD600 reaches 0.6 to 0.8, induce 6his-SmPRMT3(184-553) with 1 mM IPTG and leave the cultures to express the protein overnight.

Purification (for 3L)

1/ Pellet cells by centrifugation (4000 rpm, 45 minutes), then resuspend pellets in cold 200 mL Lysis buffer. On ice, sonicate cell suspension 3 times for 5 minutes (amplitude 60, frequency 0.5), then clarify the lysate (41657G, 45 minutes, 4°C).

2/ Incubate crude extract with 5 mL of equilibrated Talon[®] metal-affinity resin for 2h, 4°C under soft agitation. Spin down beads (233G, 2 minutes, 4°C) and discard supernatant. *store 5 μL for SDS-PAGE analysis.* resuspend beads in 10 mL cold Talon wash buffer and load them on a clean column. Wash the resin with 40 mL Talon wash buffer (Total = 50 mL = 10V column). *store 10 μL for SDS-PAGE analysis.* In the column, incubate the resin in 7 mL cold Talon elution buffer, 15 minutes, 4°C under soft agitation, then store the eluate on ice. Repeat this step once then pass the remaining buffer through the column to get remaining eluate. *store 5 μL for SDS-PAGE analysis.* Regenerate the column with 20 mM MES pH5, 300 mM NaCl. *store 10 μL for SDS-PAGE analysis.*

3/

Buffers

Lysis buffer (200 mL): 50 mM BTP pH9, 250 mM NaCl, 1 mM TCEP, 0.01% NP40, Complete[®]-EDTA free protease inhibitor cocktail (1 pill / 50 mL)

Talon wash buffer (100 mL): 50 mM BTP pH9, 250 mM NaCl, 1 mM TCEP, 10 mM imidazole

Talon elution buffer (20 mL): 50 mM BTP pH9, 250 mM NaCl, 1 mM TCEP, 150 mM imidazole

Dilution buffer (180 mL): 20 mM BTP pH9, 1 mM TCEP, 1 mM EDTA

ANX buffer (200 mL): 20 mM BTP pH9, 25 mM NaCl, 1 mM TCEP, 1 mM EDTA

Gradient buffer (100 mL): 20 mM BTP pH9, 1 M NaCl, 1 mM TCEP, 1 mM EDTA

GF buffer (350 mL): 20 mM BTP pH9, 50 mM NaCl, 1 mM TCEP, 1 mM EDTA

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