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

Construct

MGSSHHHHHHSSGTGSGENLYFQGHMSPCDQSSNYDLDMEPNDSSYFGSYGHFEIHGEMINDRVRTESYVNFILSN
AEKYFKHKIILDVGSGSGILSIIAAQAGASHVYGVEAADEIYAASHETLRVNNLLERVTFIHGQAESVELPVKKVDVIISE
WMGYFLFFESMLDSVLKMASKYLSRDGHIFPRHYTLNLLGVQCSEQLRKRRLEHWNNVYGYNMPALRRAALSEAHV
LNLTNEHVTPPISPITILTQSFELVALDLDDMHRNRIYNLSNHCSLLCEQKFHLTIQPTTDINNNSSSSSYELDAIVGYFD
VRFDDDADCKVEFSTSPTTPLTHWKQTLLFLDKPIRVKPGDKISGIITIRRATTDNRGLEINLLIGETENSLEIKQTFDLIG

Length = 396 AA

Molecular weight = 44782.3 Da

pI 5.66

ξ(red) 40340 $L.M^{-1}$

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 innoculate liquide **2X** LB medium + 100 $\mu g/mL^{-1}$ 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)

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). Incubate crude extract with 5 mL of equilibrated Talon®<sup/> 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). **Buffers**

Lysis buffer (200 mL): 50 mM BTP pH9, 250 mM NaCl, 1 mM TCEP, 0.01% NP40, Complete<sup>®-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