

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

### **Construct**

MGSSHHHHHHSSGTGSGENLYFQGHMSPCDQSSNYDLDMEPNDSSYFGSYGHFEIHGEMINDRVRTESYVNFILSN  
AEKYFKHKIILDVGS GSGILSIIAAQAGASHVYGVEAADEIYAASHETLRVNNLLERVTFIHGQAESVELPVKKVDVIISE  
WMGYFLFFESMLDSVLKMASKYLSRDGHIFPRHYTLNLLGVQCSEQLRKRRLHWNVYGYNMPALRRAALSEAHV  
LNLNEHVTPPISPITILTQSFELVALDLDDMHRNRIYNLSNHCSLLCEQKFHLTIQPTTDINNNSSSSSYELDAIVGYFD  
VRFDDADCKVEFSTSPPTPLTHWKQTLFLDKPIRVKPGDKISGIITIRRATTDNRGLEINLLIGETENSLEIKQTFDLIG

Length = 396 AA

Molecular weight = 44782.3 Da

pI 5.66

$\xi(\text{red})$  40340 L.M<sup>-1</sup>

### **Expression**

Grow p<sub>nEAvH\_SmPRMT3-184</sub> 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 µg/mL<sup>-1</sup> Ampicilline. Grow culture(s) at 37°C, 200 rpm and measure OD<sub>600</sub> every 20 minutes. When OD<sub>600</sub> reaches 0.4, set temperature to 20°C. When OD<sub>600</sub> 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<sup>®</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).

**3/**

### **Buffers**

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

From:  
<https://bsi.inscog.eu/> - **BSI wiki**

Permanent link:  
[https://bsi.inscog.eu/doku.php?id=purification:expression\\_and\\_purification\\_of\\_smprmt3\\_184-553&rev=1477663266](https://bsi.inscog.eu/doku.php?id=purification:expression_and_purification_of_smprmt3_184-553&rev=1477663266)

Last update: **2023/11/01 20:17**

