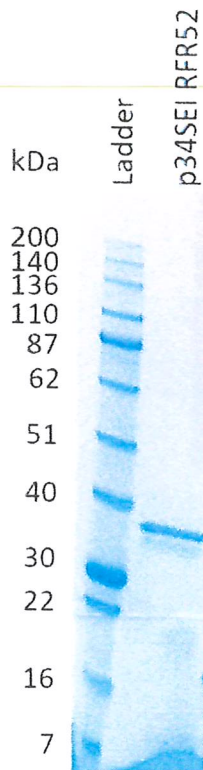


17 July 2020

SDS PAGE Analysis



- Novex Wedgewell 10-20% Tris-glycine 12 well gel (Invitrogen, XP10202BOX), reducing gel
- Ladder: 3 μ L RunBlue TriColour pre-stained (Expedeon, NXA6050)
- 1.3 μ g of protein loaded
- Protein purity >90%

Peptide Mapping of Purified band using SCIEX x500b

The bands were reduced, alkylated and digested with trypsin and chymotrypsin. The resultant peptides were run on the Exion LC coupled to the X500B mass spectrometer, a 10 minute reversed phase gradient was used. The data was searched against Swissprot using Mascot Daemon and the sequences above were searched using the BioPharmaView software to determine coverage. Yellow highlighted sequence equates to peptides identified by mass spec.

6His-TEV-p34SEI sequence coverage = 71.3%

MGHHHHHNGENLYFQGLSKGLKREKEEKEPLAVDSWWLDPGHTAVAQAPPAVASSSLFDLSVLKHLHSLQQSEPDRLHLVLVNTLR
RIQASMAPAAALPPVPSPPAAPSVADNLLASSDAALSASMASLLEDLSHIEGLSQAPQPLADEGPPGRSIGGAAPSLGALDLLGPATGCL
LDDGLEGLFEDIDTSMYDNLWAPASEGLKPGPEDGPGKEERPELDEAEFLDYLMEDVLVGTQALERPPGPGR

Swissprot match to SRTD1_HUMAN.

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QC Document of 6His-TEV-p34SEI

Construct

6xHis-tag

TEV cleavage sequence

>6His-TEV-p34SEI (*E. coli* expression = pET28b)

MGHHHHHHGENLYFQGLSKGLKRKREEEEEKEPLAVDSWWLDPGHTAVAQAPPAVASSSLFDLSVLKLHHSIQ
QSEPDRLHLVLVVNTLRRIQASMAPAAALPPVSPSPAAPSADNLLASSDAALSASMASLLEDLSHIEGLSQAPQP
LADEGPPGRSIGGAAPSLGALDLLGPATGCLDDGLEGLFEDIDTSMYDNELWAPASEGLKPGPEDGPGKEEAP
LDEAELDYLMDVLVGTQALERPPGPGR

Number of amino acids: 251

Molecular weight: 26492.62

Theoretical pI: 4.54

Extinction coefficients:

Extinction coefficients are in units of $M^{-1} cm^{-1}$, at 280 nm measured in water.

Ext. coefficient 20970

Abs 0.1% (=1 g/l) 0.792, assuming all pairs of Cys residues form cystines

Ext. coefficient 20970

Abs 0.1% (=1 g/l) 0.792, assuming all Cys residues are reduced

Summary

6His-TEV-p34SEI

0.074 mg/mL

12 x 500 μ L (444 μ g)

Buffer: 20 mM Tris/HCl pH 8.0, 150 mM NaCl, 5% glycerol, 0.5 mM TCEP, 0.5% DDM

Batch No. RFR52

$$0.074 \text{ mg/mL} \times \frac{1 \text{ mol}}{26492 \text{ g}} \times \frac{100 \text{ mL}}{1 \text{ L}} = 2.79 \text{ }\mu\text{M}$$