

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of VDAC1 [1 – 283]

Enzyme description:- VDAC1 [1 – 283]

Clone number:- DU 30855

Source:- Recombinant

Expression system:- *E.coli*,

Tag:- N-terminal GST

Purification method:- GSH-Sepharose

Calculated molecular mass:-

Monoisotopic 57, 560.21 daltons

Average Mass 57, 596.76 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.54

Purity:- >80 %

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.03 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

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Clone Data Sheet

VDAC1 [1 - 283]

<u>Protein</u>	VDAC1 [1 - 283]
<u>Clone number</u>	DU 30855
<u>Species</u>	Human
<u>Accession number</u>	P21796.2
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed VDCA1 protein</u>	MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAETSMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY LKSSKYIAWPLQGWAQTFGGGDHPPKSDLEVLFGQPLGSMVPPTYAD LGKSARDVFTKGYGFGLIKLDLKTKESENLEFTSSGSANTETTKVTGS LETKYRWTEYGLTFTEKWNTDNTLGTEITVEDQLARGLKLTFDSSFSP NTGKKNAKIKTGKREHINLGCMDMFDIAGPSIRGALVLGYEGWLAGY QMNFETAKSRVTQSNFAVGKYKTDEFQLHTNVNDGTEFGGSIYQKVNKK LETAVNLAWTAGNSNTRFGIAAKYQIDPDACFSKVNNSSLIGLGYTQ TLKPGIKLTLSALLDGKNVNAGGHKLGLGLEFQA
<u>Native sequence</u>	Amino acids M1 – A283 (end) of human VDAC1. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 - 220.
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGP</u>) residues 221 - 229
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> I sites of pGEX6P1

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Nucleotide

Sequence Of Insert

ggatccATGGCTGTGCCACCCACGTATGCCGATCTTGGCAAATCTGCC
AGGGATGTCTTCACCAAGGGCTATGGATTTGGCTTAATAAAGCTTGAT
TTGAAAACAAAATCTGAGAATGGATTGGAATTTACAAGCTCAGGCTCA
GCCAACACTGAGACCACCAAAGTGACGGGCAGTCTGGAAACCAAGTAC
AGATGGACTGAGTACGGCCTGACGTTTACAGAGAAATGGAATACCGAC
AATACACTAGGCACCGAGATTACTGTGGAAGATCAGCTTGCACGTGGA
CTGAAGCTGACCTTCGATTCATCCTTCTCACCTAACACTGGGAAAAAA
AATGCTAAAATCAAGACAGGGTACAAGCGGGAGCACATTAACCTGGGC
TGCGACATGGATTTTCGACATTGCTGGGCCTTCCATCCGGGGTGCTCTG
GTGCTAGGTTACGAGGGCTGGCTGGCCGGCTACCAGATGAATTTTGAG
ACTGCAAAATCCCAGGTGACCCAGAGCAACTTTGCAGTTGGCTACAAG
ACTGATGAATTCCAGCTTCACACTAATGTGAATGACGGGACAGAGTTT
GGCGGCTCCATTTACCAGAAAGTGAACAAGAAGTTGGAGACCGCTGTC
AATCTTGCCTGGACAGCAGGAAACAGTAACACGCGCTTCGGAATAGCA
GCCAAGTATCAGATTGACCCTGACGCCTGCTTCTCGGCTAAAGTGAAC
AACTCCAGCCTGATAGGTTTAGGATACACTCAGACTCTAAAGCCAGGT
ATTAAACTGACACTGTCAGCTCTTCTGGATGGCAAGAACGTCAATGCT
GGTGGCCACAAGCTTGGTCTAGGACTGGAATTTCAAGCataagcggcc
gc