Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active USP9Y [1550 – 2000]

Enzyme description:-	USP9Y [1550 - 2000]
Clone number:-	DU 21846
Source:-	Recombinant
Expression system:-	*E.coli*
Tag:-	N-terminal GST
Purification method:-	GSH Sepharose

Calculated molecular mass:-

Monoisotopic	79, 127.15 daltons
Average Mass	79, 178.14 daltons

[cysteines reduced, methionines have not been oxidised

Theoretical pI:-	5.34
Purity:-	80 %
Activation protocol:-	Constitutively active

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:-	-70 °C

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Clone Data Sheet

USP9Y [1550 - 2000]

Protein	USP9Y [1550 - 2000]
Clone number	DU 21846
Species	Human
Accession number	NM_004654.4
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**VGPRPPKGFVGLKN** **AGATCYMNSVIQQLYMIPSIRNSILAIEGTGSDLHDDMFGDEKQDSESN** **VDPRDDVFGYPHQFEDKPALSKTEDRKEYNIGVLRHLQVIFGHLAASQL** **QYYVPRGFWKQFRLWGEPVNLREQHDALEFFNSLVDSLDEALKALGHPA** **ILSKVLGGSFADQKICQGCPHRYECEESFTTLNVDIRNHQNLLDSLEQY** **IKGDLLEGANAYHCEKCDKKVDTVKRLLIKKLPRVLAIQLKRFDYDWER** **ECAIKFNDYFEFPRELDMGPYTVAGVANLERDNVNSENELIEQKEQSDN** **ETAGGTKYRLVGVLVHSGQASGGHYYSYIIQRNGKDDQTDHWYKFDDGD** **VTECKMDDDEEMKNQCFGGEYMGEVFDHMMKRMSYRRQKRWWNAYILFY** **EQMDMIDEDDEMIRYISELTIARPHQIIMSPAIERSVRKQNVKFM**
Native sequence	Amino acids V1550 – M2000 (end residue is Q2555) of human USP9Y. Residue V232 of the fusion protein is equivalent to V1550 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission (LEVLFQGP) residues 221 - 229
Cloning sites	*Bam*H1 and *Not*1 sites of pGEX 6P-1

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Nucleotide Sequence of insert

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ggatccGTTGGACCCCGCCCACCAAAAGGATTTGTGGGACTCAAAAATG
CTGGTGCTACGTGTTACATGAACTCTGTGATCCAGCAGCTATACATGAT
TCCTTCTATCAGGAACAGTATTCTTGCAATTGAAGGCACAGGTAGTGAT
TTACACGATGATATGTTCGGGGATGAGAAGCAGGACAGTGAGAGTAATG
TTGATCCCCGAGATGATGTATTTGGATATCCTCATCAATTTGAAGACAA
GCCAGCATTAAGTAAGACAGAAGATAGGAAAGAGTATAATATTGGTGTC
CTAAGACACCTTCAGGTCATCTTTGGTCATTTAGCTGCTTCCCAACTAC
AATACTATGTACCCAGAGGATTTTGGAAACAGTTCAGGCTTTGGGGTGA
ACCTGTTAATCTCCGTGAACAACATGATGCCTTAGAGTTTTTTAATTCT
TTGGTGGATAGTTTAGATGAAGCTTTAAAAGCTTTAGGACACCCGGCTA
TACTAAGTAAAGTCCTAGGAGGCTCCTTTGCTGATCAGAAGATCTGCCA
AGGCTGCCCACATAGGTATGAATGTGAAGAATCTTTTACAACTTTGAAT
GTGGATATTAGAAATCATCAAAATCTTCTTGACTCTTTGGAACAGTATA
TCAAAGGAGATTTATTGGAAGGTGCAAATGCATATCATTGTGAAAAATG
TGATAAAAAGGTTGACACAGTAAAGCGCCTGCTAATTAAAAAATTGCCT
CGGGTTCTTGCTATCCAACTCAAACGATTTGACTATGACTGGGAAAGAG
AATGTGCAATTAAATTCAATGATTATTTTGAATTTCCTCGAGAGCTGGA
TATGGGACCTTACACAGTAGCAGGTGTTGCAAACCTGGAAAGGGATAAT
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AACAAATGGATATGATAGATGAAGATGATGAGATGATAAGATACATATC
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GAGAGAAGTGTACGGAAACAAAATGTGAAATTTATGtaagcggccgc
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