

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of PFKFB3 [1 – 520]

Enzyme description:- PFKFB3 [1 – 520]

Clone number:- DU 36863

Source:- Recombinant

Expression system:- *E.coli*,

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 86, 377.86 daltons

Average Mass 86, 433.32 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 7.50

Purity:- >80 %

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

PFKFB3 [1 – 520]

<u>Protein</u>	PFKFB3 [1 – 520]
<u>Clone number</u>	DU 36863
<u>Species</u>	Human
<u>Accession number</u>	NM_004566.3
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAETSMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLVLFQGPLGSMPLELTQSRVQKIW VPVDHRPSLPRSCGPKLTNSPTVI VMVGLPARGKTYISKKLTRYLNWIG VPTKVFNVGEYRREAVKQYSSYNFFRPDNEEAMKVRKQCALAALRDVKS YLAKEGGQIAVFDATNTTRERRHMILHFAKENDFKAFFIESVCDDPTV ASNIMEVKISSPDYKDCNSAEAMDDFMKRISCYEASYQPLDPDKCDRDL SLIKVIDVGRRFLVNRVQDHIQSRIVYVYLMNIHVQPTIYLCRHHGENEH NLQGRIGGDSGLSSRGKFFASALS K FVEEQNLKDLRVWTSQLKSTIQTA EALRLPYEQWKALNEIDAGVCEELTYEEIRDTYPEEYALREQDKYYYRY PTGESYQDLVQRLPEVMELERQENVLVICHQAVLRCLLAYFLDKSAEE MPYLKCP LHTVLKLT P VAYGCRVESIYLNVESVCTHRESEDAKKGPNP LMRRNSVTPLASPEPTKKPRINSFEEHVASTSAALPSCLPPEVPTQLPG QNMKGSRSSADSSRKH</p>
<u>Native sequence</u>	<p>Amino acids M1 – H520 (end) of human PFKFB3. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.</p>
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGP</u>) residues 221 - 228
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> 1 sites into pGEX6P-3

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Nucleotide Sequence of Insert:

ggatccATGCCGTTGGAAGTACGACGAGCCGAGTGCAGAAGATCTGGGT
GCCCCGTGGACCACAGGCCCTCGTTGCCAGATCCTGTGGGCCAAAGCTGA
CCAATCCCCACCGTCATCGTCATGGTGGGCCTCCCCGCCGGGGCAAG
ACCTACATCTCCAAGAAGCTGACTCGCTACCTCAACTGGATTGGCGTCCC
CACAAAAGTGTTCACGTCGGGGAGTATCGCCGGGAGGCTGTGAAGCAGT
ACAGCTCCTACAACCTTCTTCCGCCCCGACAATGAGGAAGCCATGAAAGTC
CGGAAGCAATGTGCCTTAGCTGCCTTGAGAGATGTCAAAGCTACCTGGC
GAAAGAAGGGGGACAAATTGCGGTTTTTCGATGCCACCAATACTACTAGAG
AGAGGAGACACATGATCCTTCATTTTGCCAAAGAAAATGACTTTAAGGCG
TTTTTCATCGAGTCGGTGTGCGACGACCCTACAGTTGTGGCCTCCAATAT
CATGGAAGTTAAAATCTCCAGCCCCGATTACAAAGACTGCAACTCGGCAG
AAGCCATGGACGACTTCATGAAGAGGATCAGTTGCTATGAAGCCAGCTAC
CAGCCCCTCGACCCCGACAAATGCGACAGGGACTTGTGCTGATCAAGGT
GATTGACGTGGGCCGGAGTTTCTGGTGAACCGGGTGCAGGACCACATCC
AGAGCCGCATCGTGTACTACCTGATGAACATCCACGTGCAGCCGCGTACC
ATCTACCTGTGCCGGCAGGGCAGAACGAGCACAACCTCCAGGGCCGCAT
CGGGGGCGACTCAGGCCGTCCAGCCGGGGCAAGAAGTTTGCCAGTGCTC
TGAGCAAGTTCGTGGAGGAGCAGAACCTGAAGGACCTGCGCGTGTGGACC
AGCCAGCTGAAGAGCACCATCCAGACGGCCGAGGCGCTGCGGCTGCCCTA
CGAGCAGTGGAAGGCGCTCAATGAGATCGACGCGGGCGTCTGTGAGGAGC
TGACCTACGAGGAGATCAGGGACACCTACCCTGAGGAGTATGCGCTGCGG
GAGCAGGACAAGTACTATTACCGTACCCACCGGGGAGTCTTACCAGGA
CCTGGTCCAGCGCTTGGAGCCAGTGATCATGGAGCTGGAGCGGCAGGAGA
ATGTGCTGGTCATCTGCCACCAGGCCGTCTGCGCTGCCTGCTTGCCCTAC
TTCCTGGATAAGAGTGCAGAGGAGATGCCCTACCTGAAATGCCCTCTTCA
CACCGTCTGAAACTGACGCCTGTGCTTATGGCTGCCGTGTGGAATCCA
TCTACCTGAACGTGGAGTCCGTCTGCACACACCGGGAGAGGTCAGAGGAT
GCAAAGAAGGGACCTAACCCGTCATGAGACGCAATAGTGTACCCCGCT
AGCCAGCCCCGAACCCACAAAAAGCCTCGCATCAACAGCTTTGAGGAGC
ATGTGGCCTCCACCTCGGCCGCCCTGCCAGCTGCCTGCCCCGGAGGTG
CCCACGCAGCTGCCTGGACAAAACATGAAAGGCTCCCGGAGCAGCGCTGA
CTCCTCCAGGAAACACtgagcggccgc

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