

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of PAX5 [1 – 391]

<u>Enzyme description:-</u>	PAX5 [1 - 391]
<u>Clone number:-</u>	DU 36786
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	<i>E.coli</i>
<u>Tag:-</u>	N-terminal GST
<u>Purification method:-</u>	GSH Sepharose

Calculated molecular mass:-

Monoisotopic 68, 929.85 daltons
Average Mass 68, 973.36 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 7.62

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

PAX5 [1 - 391]

<u>Protein</u>	PAX5 [1 - 391]
<u>Clone number</u>	DU 36786
<u>Species</u>	Human
<u>Accession number</u>	NM_016734.2
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFFQGPLGSMDLEKNYPTPRTSR TGHGGVNQLGGVFNVRPLPDVVRQRIVELAHQGVRPCDISRQLRVSHG CVSKILGRYYETGSIKPGVIGGSKPKVATPKVVEKIAEYKRQNPTMFAW EIRDRLLAERVCDNDTVPSVSSINRIIRTKVQQPPNQPVPASSHSIVST GSVTQVSSVSTDSAGSSYSISGILGITSADTNKRKRDEGIQESVVPN GHSLPGRDFLRKQMRGDLFTQQQLEVLDRVFERQHYSDFTTTEPIKPE QTTEYSAMASLAGGLDDMKANLASPTPADIGSSVPGPQSYPIVTGRDLA STTLPGYPPHVPPAGQGSYSAPTTLTGMVPGSEFSGSPYSHPOYSSYNDS WRFPNPGLLGSPYYSAAARGAAPPAAATAYDRH</p>
<u>Native sequence</u>	<p>Amino acids M1 – H391 (end) of human PAX5. 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>Bg</i> III and <i>Not</i> I into <i>Bam</i> H1 and <i>Not</i> I sites of pGEX6P-1

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Nucleotide Sequence of insert

agatctATGGATTTAGAGAAAAATTATCCGACTCCTCGGACCAGCAGGAC
AGGACATGGAGGAGTGAATCAGCTTGGGGGGTTTTTGTGAATGGACGGC
CACTCCCGGATGTAGTCCGCCAGAGGATAGTGGAAGTTGCTCATCAAGGT
GTCAGGCCCTGCGACATCTCCAGGCAGCTTCGGGTCAGCCATGGTTGTGT
CAGCAAATTCTTGGCAGGTATTATGAGACAGGAAGCATCAAGCCTGGGG
TAATTGGAGGATCCAAACCAAAGGTCGCCACACCCAAAGTGGTGGAAAAA
ATCGCTGAATATAAACGCCAAAATCCCACCATGTTTGCCTGGGAGATCAG
GGACCGGCTGCTGGCAGAGCGGGTGTGTGACAATGACACCGTGCTAGCG
TCAGTTCCATCAACAGGATCATCCGGACAAAAGTACAGCAGCCACCCAAC
CAACCAGTCCCAGCTTCCAGTCACAGCATAGTGTCCACTGGCTCCGTGAC
GCAGGTGTCCTCGGTGAGCACGGATTCCGGCCGGCTCGTCGTA TCCATCA
GCGGCATCCTGGGCATCACGTCCCCCAGCGCCGACACCAACAAGCGCAAG
AGAGACGAAGGTATTCAGGAGTCTCCGGTGCCGAACGGCCACTCGCTTCC
GGCAGAGACTTCCTCCGGAAGCAGATGCGGGGAGACTTGTTACACACAGC
AGCAGCTGGAGGTGCTGGACCGCGTGTTTGAGAGGCAGCACTACTCAGAC
ATCTTCACCACCACAGAGCCCATCAAGCCCGAGCAGACCACAGAGTATTC
AGCCATGGCCTCGCTGGCTGGTGGGCTGGACGACATGAAGGCCAATCTGG
CCAGCCCCACCCCTGCTGACATCGGGAGCAGTGTGCCAGGCCCGCAGTCC
TACCCCATTTGTGACAGGCCGTGACTTGCCGAGCACGACCCTCCCCGGGTA
CCCTCCACACGTCCCCCGCTGGACAGGGCAGCTACTCAGCACCGACGC
TGACAGGGATGGTGCCTGGGAGTGAGTTTTCCGGGAGTCCCTACAGCCAC
CCTCAGTATTCCCTCGTACAACGACTCCTGGAGGTTCCCAACCCGGGGCT
GCTTGGCTCCCCCTACTATTATAGCGCTGCCGCCCGAGGAGCCGCCCCAC
CTGCAGCCGCCACTGCCATGACCGTCACTgagcggccgc

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