

Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active KIT [544 - 976]

<u>Enzyme description:-</u>	KIT [544 - 976]
<u>Clone number:-</u>	DU 9182
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal His(6) tag
<u>Purification method:-</u>	Ni ²⁺ -NTA agarose

Calculated molecular mass:-

Monoisotopic 52, 331.96 daltons
Average Mass 52, 365.85 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.50

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,
10 mM DTT, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 °C

Assay Buffer:-

50 mM Tris-HCl pH 7.5, 0.1mM EGTA, 10 mM DTT, 10 mM MgAc

Substrate:-

GGMEDIYFEFMGGKKK Final concentration: 300 uM

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Clone Data Sheet

KIT [544 - 976]

Protein KIT [544 - 976]

Clone number DU 9182

Species Human

Accession number NM_000222.2

Tags N-terminal His6

Bacterially expressed protein
MSYYHHHHHDYDIPPTENLYFQGAMGSTYKYLQKPMYEVQWKVV
EEINGNNYVYIDPTQLPYDHKWEFPNRNLSFGKTLGAGAFGVVVE
ATAYGLIKSDAAMTVAVKMLKPSAHLTEREALMSELKVLSYLGHN
MNIVNLLGACTIGGPTLVITEYCCYGDLLNFLRRKRDSFICSKQE
DHAEAALYKNLLHSKESSCSDSTNEYMDMKPGVSYVVPTKADKRR
SVRIGSYIERDVTPAIMEDDELALDLEDLLSFSYQVAKGMAFLAS
KNCIHRDLAARNILLHGRITKICDFGLARDIKNDSNYVVKGNA
LPVKWMAPESiFNCVYTFESDVWSYGYIFLWELFSLGSSPYPGMPV
DSKFYKMIKEGFRMLSPEHAPAEMYDIMKTCWDADPLKRPTFKQI
VOLIEKQISESTNHIYSNLANCSPNQRQPKVVDHSVRINSGSTAS
SSQPLLVHDDV

Native sequence Amino acids T544 – V976 (end) of human KIT.

ResidueT29 of the fusion protein is equivalent to T544 of the native enzyme. The His6 tag is located at residues 5 - 10.

Protease cleavage rTEV (ENLYFQG) residues 18 - 24

Cloning sites *Bam*H1 and *Eco*R1sites of pFastBac HTb.

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<u>Nucleotide sequence of insert</u>	ggatccACCTACAAATATTACAGAAACCCATGTATGAAGTACAG TGGAAAGGTTGTTGAGGAGATAAATGGAAACAATTATGTTACATA GACCCAACACAACTTCCTATGATCACAAATGGGAGTTCCAGA AACAGGCTGAGTTGGGAAAACCCCTGGGTCTGGAGCTTCAGG AAGGTTGTTGAGGCAACTGCTTATGGCTTAATTAAGTCAGATGCG GCCATGACTGTCGCTGTAAAGATGCTCAAGCCGAGTGCCCATTG ACAGAACGGGAAGCCCTCATGTCTGAACCAAAGTCTGAGTAC CTTGGTAATCACATGAATATTGTGAATCTACTTGGAGCCTGCACC ATTGGAGGGCCACCCCTGGTCATTACAGAATATTGTTGCTATGGT GATCTTTGAATTGGAGAAGAAAACGTGATTCAATTATTGTT TCAAAGCAGGAAGATCATGCAGAAGCTGCACCTTATAAGAATCTT CTGCATTCAAAGGAGTCTTCCTGCAGCGATAGTACTAATGAGTAC ATGGACATGAAACCTGGAGTTCTTATGTTGTCACCAAGGCC GACAAAAGGAGATCTGTGAGAATAGGCTACATAGAAAGAGAT GTGACTCCGCCATCATGGAGGATGACGAGTTGGCCCTAGACTA GAAGACTGCTGAGCTTTCTTACCAAGGTGGCAAAGGGCATGGCT TTCCTCGCCTCCAAGAATTGTATTACAGAGACTTGGCAGCCAGA AATATTCTCCTTACTCATGGTCGGATCACAAAGATTGTGATT GGTAGGCCAGAGACATCAAGAATGATTCCAATTATGTGGTTAAA GGAAACGCTCGACTACCTGTGAAGTGGATGGCACCTGAAAGCATT TTCAACTGTGTATACACGTTGAAAGTGACGTCTGGCCTATGGG ATTTTCCTTGGGAGCTGTTCTTTAGGAAGCAGCCCTATCCT GGAATGCCGTCGATTCTAAGTTACAAGATGATCAAGGAAGGC TTCCGGATGCTCAGCCCTGAACACCGCACCTGCTGAAATGTATGAC ATAATGAAGACTTGCTGGGATGCAAGATCCCTAAAAAGACCAACA TTCAAGCAAATTGTTCAGCTAATTGAGAAGCAGATTTCAGAGAGC ACCAATCATATTACTCCAACCTAGCAAACCTGCAAGCCCCAACCGA CAGAAGCCCCGTGGTAGACCATTCTGTGCGGATCAATTCTGTGGC AGCACCGCTTCCTCCTCCCAGCCTTGCTGTGCACGACGATGTC tgagaattc
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