

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active B-Raf V600E [2 – 766]

<u>Enzyme description:-</u>	B-Raf V600E [2 – 766]
<u>Clone number:-</u>	DU 15485
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal GST
<u>Purification method:-</u>	GSH Sepharose

Calculated molecular mass:-

Monoisotopic	111,757.49 daltons
Average Mass	111,828.55 daltons
[cysteines reduced, methionines have not been oxidised]	

Theoretical pI:- 6.53

Purity:- 85 %

Enzyme storage buffer:-

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

Storage temperature:- -70°C

Assay buffer:-

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

Substrate:-

Three step assay in which B-Raf activates inactive MKK1, which in turn activates inactive p42MAPKinase. Activity of p42MAPKinase is then assayed against myelin basic protein as substrate (final concentration of 0.3 mg/ml), in a standard filter binding assay.

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Clone Data Sheet

B-Raf V600E [2 – 766]

<u>Protein</u>	B-Raf V600E [2 – 766]
<u>Clone number</u>	DU 15485
<u>Species</u>	Human
<u>Accession number</u>	NM_004333.4
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKK FELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMMLGGCPKERA EISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFED RLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFK KRIEAIPOIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLEVL QGPLGSPNSRVDAALSGGGGGGAEPGQALFNGDMEPEAGAGAGAA ASSAADPAIPEEVWNIQMIKLTQEHIEALLDKFGGEHNPPSIYL EAYEYTSKLDALQOREQQLLESLGNGTDFSVSSSASMDTVTSSS SSSLSVLPSSLSVFQNPTDVARSNPKSPQKPIVRVFLPNKQRTVV PARCGVTVRDSLKKALMMRGLIPECCAVYRIQDGEKKPIGWDTDI SWLTGEELHVEVLENVPLTTHNFVRKTFFTLAFCDFCRKLLFQGF RCQTCGYKFHQRCSTEVPLMCVNYDQLDLLFVSKFFEHHPIPQEE ASLAETALTSGSSPSAPASDSIGPQILTSPSPSKSIPIPQPFRPA DEDHRNQFGQDRSSAPNVHINTIEPVNIDDLIRDQGFRGDGGS TTGLSATPPASLPGSLTNVKALQKSPGPQRERKSSSSSEDRNRMK TLGRRDSSDDWEIPDGQITVGQRIGSGSFGTVYKGKWHGDVAVKM LNVTAPTPQQLQAFKNEVGVLRKTRHVNILLFMGYSTKPQLAIVT QWCEGSSLYHHLHIETKFEMIKLIDIARQTAQGMDYLHAKSIIH RDLKSNNIFLHEDLTVKIGDFGLATEKSRWSGSHQFEQLSGSILW MAPEVIRMQDKNPYSFQSDVYAFGIVLYELMTGQLPYSNINNRDQ IIFMVGRGYLSPDLSKVRSNCPKAMKRLMAECLKKRDERPLFPQ ILASIELLARSLPKIHRSASEPSLNRAGFQTEDFSLYACASPKTP IQAGGYGAFPVH
<u>Native sequence</u>	Amino acids A2 – H766 (end) of human B-Raf. Residue A238 of the fusion protein is equivalent to A2 of the native

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enzyme. The GST tag is located at residues 1 - 220.

The enzyme has a V600E mutation to mimic the mutation found in 66 % of malignant melanomas. Residue E600 is equivalent to E836 of the fusion protein.

Protease cleavage

PreScission (LEVLFQGP) residues 221 - 228

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Nucleotide
sequence of insert

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gtcgacGCGGCGCTGAGCGGTGGCGGTGGTGGCGGCGCGGAGCCG
GGCCAGGCTCTGTTCAACGGGGACATGGAGCCCGAGGCCGGCGCC
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GAGGAGGTGTGGAATATCAAACAAATGATTAAAGTTGACACAGGAA
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CAGAGGACAGTGGTACCTGCAAGGTGTGGAGTTACAGTCCGAGAC
AGTCTAAAGAAAGCACTGATGATGAGAGGTCTAATCCAGAGTGC
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