Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active B-Raf [2 – 766]

Enzyme description:- B-Raf [2 – 766]

Clone number:- DU 15484

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 111, 727.51 daltons
Average Mass 111, 798.57 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.62

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 unactive MKK1, which in turn activates unactive 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.

Clone Data Sheet

B-Raf [2 – 766]

Protein	B-Raf [2 – 766]
Clone number	DU 15484
Species	Human
Accession number	NM_004333.4
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKK FELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERA EISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFED RLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFK KRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLF QGPLGSPNSRVD**AALSGGGGGGAEPGQALFNGDMEPEAGAGAGAA** **ASSAADPAIPEEVWNIKQMIKLTQEHIEALLDKFGGEHNPPSIYL** **EAYEEYTSKLDALQQREQQLLESLGNGTDFSVSSSASMDTVTSSS** **SSSLSVLPSSLSVFQNPTDVARSNPKSPQKPIVRVFLPNKQRTVV** **PARCGVTVRDSLKKALMMRGLIPECCAVYRIQDGEKKPIGWDTDI** **SWLTGEELHVEVLENVPLTTHNFVRKTFFTLAFCDFCRKLLFQGF** **RCQTCGYKFHQRCSTEVPLMCVNYDQLDLLFVSKFFEHHPIPQEE** **ASLAETALTSGSSPSAPASDSIGPQILTSPSPSKSIPIPQPFRPA** **DEDHRNQFGQRDRSSSAPNVHINTIEPVNIDDLIRDQGFRGDGGS** **TTGLSATPPASLPGSLTNVKALQKSPGPQRERKSSSSSEDRNRMK** **TLGRRDSSDDWEIPDGQITVGQRIGSGSFGTVYKGKWHGDVAVKM** **LNVTAPTPQQLQAFKNEVGVLRKTRHVNILLFMGYSTKPQLAIVT** **QWCEGSSLYHHLHIIETKFEMIKLIDIARQTAQGMDYLHAKSIIH** **RDLKSNNIFLHEDLTVKIGDFGLATVKSRWSGSHQFEQLSGSILW** **MAPEVIRMQDKNPYSFQSDVYAFGIVLYELMTGQLPYSNINNRDQ** **IIFMVGRGYLSPDLSKVRSNCPKAMKRLMAECLKKKRDERPLFPQ** **ILASIELLARSLPKIHRSASEPSLNRAGFQTEDFSLYACASPKTP** **IQAGGYGAFPVH**
Native sequence	Amino acids A2 – H766 (end) of human B-Raf. Residue A238 of the fusion protein is equivalent to A2 of the native

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

Protease cleavage PreScission (LEVLFQGP) residues 221 - 228

Nucleotide sequence of insert

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gtcgacGCGGCGCTGAGCGGTGGCGGTGGTGGCGGCGCGGAGCCG
GGCCAGGCTCTGTTCAACGGGGACATGGAGCCCGAGGCCGGCGCC
GGCGCCGGCGCCGCGGCCTCTTCGGCTGCGGACCCTGCCATTCCG
GAGGAGGTGTGGAATATCAAACAAATGATTAAGTTGACACAGGAA
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