Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of ERK2 D168A [2 - 360]

Enzyme description:- ERK2 D168A [2 – 360]

Clone number:- DU 663

Source:- Recombinant

Expression system:- *E.coli* expression vector system

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 68, 620.18 daltons
Average Mass 68, 664.40 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.36

Purity:- >80 %

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM Sucrose, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Assay buffer:-
50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1 % 2–mercaptoethanol, 10 mM MgAc

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Clone Data Sheet

ERK2 D168A [2 - 360]

Protein	ERK2 D168A [2 - 360]
Clone number	DU 663
Species	Human
Accession number	NM_002745
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQPLGSPNSRVD**AAAA** **AAGAGPEMVRGQVFDVGPRYTNLSYIGEGAYGMVCSAYDNVNKVRVAI** **KKISPFEHQTYCQRTLREIKILLRFRHENIIGINDIIRAPTIEQMKDV** **YIVQDLMETDLYKLLKTQHLSNDHICYFLYQILRGLKYIHSANVLHRD** **LKPSNLLLNTTCDLKICAFGLARVADPDHDHTGFLTEYVATRWYRAPE** **IMLNSKGYTKSIDIWSVGCILAEMLSNRPIFPGKHYLDQLKHILGILG** **SPSQEDLNCIINLKARNYLLSLPHKNKVPWNRLFPNADSKALDLLDKM** **LTFNPHKRIEVEQALAHPYLEQYYDPSDEPIAEAPFKFDMELDDLPKE** **KLKELIFEETARFQPGYRS**
Native sequence	Amino acids A2 – S360 (end) of human ERK2. Residue A237 of the fusion protein is equivalent to A2 of the native enzyme. The GST tag is located at residues 1 – 220. The enzyme has a D168A mutation, which produces a kinase dead enzyme. Residue D168A is equivalent to A402 of the fusion protein.
Protease cleavage	PreScission site (LEVLFQGP) residues 221 – 228
Cloning sites	*Sal*1 and *Not*1 sites of pGex6P1

Nucleotide sequence of insert

gtcgacGCGGCGGCGGCGGCGGGCGCGGGCCCGGAGATGGTCCGC
GGGCAGGTGTTCGACGTGGGGCCGCGCTACACCAACCTCTCGTACATC
GGCGAGGGCGCCTACGGCATGGTGTGCTCTGCTTATGATAATGTCAAC
AAAGTTCGAGTAGCTATCAAGAAAATCAGCCCCTTTGAGCACCAGACC
TACTGCCAGAGAACCCTGAGGGAGATAAAAATCTTACTGCGCTTCAGA
CATGAGAACATCATTGGAATCAATGACATTATTCGAGCACCAACCATC
GAGCAAATGAAAGATGTATATATAGTACAGGACCTCATGGAAACAGAT
CTTTACAAGCTCTTGAAGACACAACACCTCAGCAATGACCATATCTGC
TATTTTCTCTACCAGATCCTCAGAGGGTTAAAATATATCCATTCAGCT
AACGTTCTGCACCGTGACCTCAAGCCTTCCAACCTGCTGCTCAACACC
ACCTGTGATCTCAAGATCTGTGCCTTTGGCCTGGCCCGTGTTGCAGAT
CCAGACCATGATCACACAGGGTTCCTGACAGAATATGTGGCCACACGT
TGGTACAGGGCTCCAGAAATTATGTTGAATTCCAAGGGCTACACCAAG
TCCATTGATATTTGGTCTGTAGGCTGCATTCTGGCAGAAATGCTTTCT
AACAGGCCCATCTTTCCAGGGAAGCATTATCTTGACCAGCTGAAACAC
ATTTTGGGTATTCTTGGATCCCCATCACAAGAAGACCTGAATTGTATA
ATAAATTTAAAAGCTAGGAACTATTTGCTTTCTCTTCCACACAAAAAT
AAGGTGCCATGGAACAGGCTGTTCCCAAATGCTGACTCCAAAGCTCTG
GACTTATTGGACAAAATGTTGACATTCAACCCACACAAGAGGATTGAA
GTAGAACAGGCTCTGGCCCACCCATATCTGGAGCAGTATTACGACCCG
AGTGACGAGCCCATCGCCGAAGCACCATTCAAGTTCGACATGGAATTG
GATGACTTGCCTAAGGAAAAGCTCAAAGAACTAATTTTTGAAGAGACT
GCTAGATTCCAGCCAGGATACAGATCTtaagcggccgcgc