Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of hnRNP K isoform b S116A [2 - 463]

Enzyme description:- hnRNP K isoform b S116A [2 – 463]

Clone number:- DU 4075

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 7 mg/L

Calculated molecular mass:-

Monoisotopic 77, 977.17 daltons
Average Mass 78, 026.63 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.47

Purity:- 90 %

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, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 °C

Assay:- Control substrate for ERK1 or ERK2

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Clone Data Sheet

hnRNP K isoform b S116A [2 - 463]

Protein	hnRNP K isoform b S116A [2 - 463]
Clone Number	DU 4075
Species	Human
Accession number	AAH14980
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPEF**ETEQPEETFPN** **TETNGEFGKRPAEDMEEEQAFKRSRNTDEMVELRILLQSKNAGAVIGKG** **GKNIKALRTDYNASVSVPDSSGPERILSISADIETIGEILKKIIPTLEE** **GLQLPAPTATSQLPLESDAVECLNYQHYKGSDFDCELRLLIHQSLAGGI** **IGVKGAKIKELRENTQTTIKLFQECCPHSTDRVVLIGGKPDRVVECIKI** **ILDLISESPIKGRAQPYDPNFYDETYDYGGFTMMFDDRRGRPVGFPMRG** **RGGFDRMPPGRGGRPMPPSRRDYDDMSPRRGPPPPPPGRGGRGGSRARN** **LPLPPPPPPRGGDLMAYDRRGRPGDRYDGMVGFSADETWDSAIDTWSPS** **EWQMAYEPQGGSGYDYSYAGGRGSYGDLGGPIITTQVTIPKDLAGSIIG** **KGGQRIKQIRHESGASIKIDEPLEGSEDRIITITGTQDQIQNAQYLLQN** **SVKQYSGKFF**
Native sequence	Amino acids E2 – F463 (end) of human hnRNP K isoform b. Residue E235 of fusion protein is equilivalent to E2 of the native enzyme. The GST tag is located at residues 1 – 220. The protein has a S116A mutation, where S116 which is phosphorylated by ERK1 / ERK2 has been changed to A. Residue S116 is equilivalent to residue A349 of the fusion protein.
Protease cleavage	PreScission (LEVLFQGPL) residues 221 - 229
Cloning sites	*Eco*R1 and *Not*1 site of pGex6P-1

Nucleotide sequence

ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCA
CTCGACTTCTTTTGGAATATCTTGAAGAAAAATATGAAGAGCATTTGTA
TGAGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTGGGT
TTGGAGTTTCCCAATCTTCCTTATTATATTGATGGTGATGTTAAATTAA
CACAGTCTATGGCCATCATACGTTATATAGCTGACAAGCACAACATGTT
GGGTGGTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCG
GTTTTGGATATTAGATACGGTGTTTCGAGAATTGCATATAGTAAAGACT
TTGAAACTCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAAATGCTGAA
AATGTTCGAAGATCGTTTATGTCATAAAACATATTTAAATGGTGATCAT
GTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTAT
ACATGGACCCAATGTGCCTGGATGCGTTCCCAAAATTAGTTTGTTTTAA
AAAACGTATTGAAGCTATCCCACAAATTGATAAGTACTTGAAATCCAGC
AAGTATATAGCATGGCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGGTG
GCGACCATCCTCCAAAATCGGATCTGGAAGTTCTGTTCCAGGGGCCCCT
GGGATCCCCGGAATTC**GAAACTGAACAGCCAGAAGAAACCTTCCCTAAC**
ACTGAAACCAATGGTGAATTTGGTAAACGCCCTGCAGAAGATATGGAAG
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ACGCATTCTGCTTCAGAGCAAGAATGCTGGGGCAGTGATTGGAAAAGGA
GGCAAGAATATTAAGGCTCTCCGTACAGACTACAATGCCAGTGTTTCAG
TCCCAGACAGCAGTGGCCCCGAGCGCATATTGAGTATCAGTGCTGATAT
TGAAACAATTGGAGAAATTCTGAAGAAAATCATCCCTACCTTGGAAGAG
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CTCCATCTAGAAGAGATTATGATGATATGAGCCCTCGTCGAGGACCACC
TCCCCCTCCTCCCGGACGAGGCGGCCGGGGTGGTAGCAGAGCTCGGAAT
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AAAGGTGGTCAGCGGATTAAACAAATCCGTCATGAGTCGGGAGCTTCGA
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TACAGGAACACAGGACCAGATACAGAATGCACAGTATTTGCTGCAGAAC
AGTGTGAAGCAGTATTCTGGAAAGTTTTTCtaagcggccgc