Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active PKA [2 - 351]

Enzyme description:- PKA [2 - 351]

Clone number:- DU 951

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 1 mg/L

Calculated molecular mass:- 67, 239 Daltons

Purity:- >70 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 50 % glycerol, 150 mM NaCl, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -20 °C

Assay:- Standard filter binding assay

Assay buffer:-

50 mM Tris-HCl pH 7.5, 0.1% 2−mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc

Substrate:-

KEMPtide (LRRASLG) Final concentration: 30 µM

Specific activity range:- 250 – 500 U/mg

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Clone Data Sheet

PKA [2 - 351]

Protein	PKA [2 - 351]
Clone number	DU 951
Species	Human
Accession number	NM_002730
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**GNAAAAKKG** **SEQESVKEFLAKAKEDFLKKWESPAQNTAHLDQFERIKTLGTGSFGRV** **MLVKHKETGNHYAMKILDKQKVVKLKQIEHTLNEKRILQAVNFPFLVK** **LEFSFKDNSNLYMVMEYVPGGEMFSHLRRIGRFSEPHARFYAAQIVLT** **FEYLHSLDLIYRDLKPENLLIDQQGYIQVTDFGFAKRVKGRTWTLCGT** **PEYLAPEIILSKGYNKAVDWWALGVLIYEMAAGYPPFFADQPIQIYEK** **IVSGKVRFPSHFSSDLKDLLRNLLQVDLTKRFGNLKNGVNDIKNHKWF** **ATTDWIAIYQRKVEAPFIPKFKGPGDTSNFDDYEEEEIRVSINEKCGK** **EFSEF**
Native sequence	Amino acids G2 – F351 (end) of human PKA. Residue G232 of the fusion protein is residue G2 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission (LEVLFQPGL) at residues 221 – 229
Cloning sites	*Bam*H1 and *Sal*1 site of pGEX6P-1

Nucleotide sequence of insert

GGATCCGGCAACGCCGCCGCCGCCAAGAAGGGCAGCGAGCAGGAGAGC
GTGAAAGAATTCTTAGCCAAAGCCAAAGAAGATTTTCTTAAAAAATGG
GAAAGTCCCGCTCAGAACACAGCCCACTTGGATCAGTTTGAACGAATC
AAGACCCTCGGCACGGGCTCCTTCGGGCGGGTGATGCTGGTGAAACAC
AAGGAGACCGGGAACCACTATGCCATGAAGATCCTCGACAAACAGAAG
GTGGTGAAACTGAAACAGATCGAACACACCCTGAATGAAAAGCGCATC
CTGCAAGCTGTCAACTTTCCGTTCCTCGTCAAACTCGAGTTCTCCTTC
AAGGACAACTCAAACTTATACATGGTCATGGAGTACGTGCCCGGCGGG
GAGATGTTCTCACACCTACGGCGGATCGGAAGGTTCAGTGAGCCCCAT
GCCCGTTTCTACGCGGCCCAGATCGTCCTGACCTTTGAGTATCTGCAC
TCGCTGGATCTCATCTACAGGGACCTGAAGCCGGAGAATCTGCTCATT
GACCAGCAGGGCTACATTCAGGTGACAGACTTCGGTTTCGCCAAGCGC
GTGAAGGGCCGCACTTGGACCTTGTGCGGCACCCCTGAGTACCTGGCC
CCTGAGATTATCCTGAGCAAAGGCTACAACAAGGCCGTGGACTGGTGG
GCCCTGGGGGTTCTTATCTATGAAATGGCCGCTGGCTACCCGCCCTTC
TTCGCAGACCAGCCCATCCAGATCTATGAGAAGATCGTCTCTGGGAAG
GTGCGCTTCCCTTCCCACTTCAGCTCTGACTTGAAGGACCTGCTGCGG
AACCTCCTGCAGGTAGATCTCACCAAGCGCTTTGGGAACCTCAAGAAT
GGGGTCAACGATATCAAGAACCACAAGTGGTTTGCCACAACTGACTGG
ATTGCCATCTACCAGAGGAAGGTGGAAGCTCCCTTCATACCAAAGTTT
AAAGGCCCTGGGGATACGAGTAACTTTGACGACTATGAGGAAGAAGAA
ATCCGGGTCTCCATCAATGAGAAGTGTGGCAAGGAGTTTTCTGAGTTT
taggtcgac