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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	P17612
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 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	*BamH1*/*Sal1* 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