Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active AMP kinase alpha 1 catalytic subunit T172D [3 - 308]

Enzyme description:- AMP kinase alpha 1 T172D [3 - 308]

Clone Number:- DU 1713

Source:- Recombinant

Expression system:- *E. coli*

Tag:- N-terminal GST and MYC

Purification method:- GSH Sepharose

Expression level:- 2 mg/L

Calculated molecular mass:- 64, 554 daltons

Purity:- > 90 %

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, 0.2 mM PMSF, 1 mM Benzamidine

Storage temperature:- -20 °C

Assay:- Standard filter binding assay

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

Substrate:-
AMARA peptide [AMARAASAAALARRR] Final concentration: 300 μM

Specific activity range:- 60 – 120 U/mg

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Clone Data Sheet

AMP kinase alpha 1 catalytic subunit T172D [3 - 308]

Protein	AMP kinase alpha 1 T172D [3 - 308]
Clone number	DU 1713
Species	Rat
Accession number	U40819
Tags	N-terminal GST and MYC (EQKLISEEDL)
E. coli expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLVPRGSEFAMEQKLISEEDLGGG**EK** **QKHDGRVKIGHYILGDTLGVGTFGKVKVGKHELTGHKVAVKILNRQKIR** **SLDVVGKIRREIQNLKLFRHPHIIKLYQVISTPSDIFMVMEYVSGGELF** **DYICKNGRLDEKESRRLFQQILSGVDYCHRHMVVHRDLKPENVLLDAHM** **NAKIADFGLSNMMSDGEFLRDSCGSPNYAAPEVISGRLYAGPEVDIWSS** **GVILYALLCGTLPFDDDHVPTLFKKICDGIFYTPQYLNPSVISLLKHML** **QVDPMKRATIKDIREHEWFKQDLPKYLFPEDPSYSSTMIDDEALKEVCE** **KFECSEEEVL**RSITLAAARDRLD
Native sequence	Amino acids E3 - L308 of rat AMP kinase alpha 1. [Full length protein ends at residue Q548] Residue E244 of the fusion protein is equivalent to E3 of the native enzyme. The enzyme has a T172D mutation in order to mimic phosphorylation of the enzyme. Residue T172 is equilivalent to D413 of the fusion protein. The GST tag is located at residues 1 - 220 and the MYC tag is located at residues 231 - 240. The following sequence is present after the AMPK sequence, RSITLAAARDRLD, residues 550 - 562.
Protease cleavage	Thrombin (LVPRGS) residues 221 - 226
Cloning sites	*Bam*H1 and *Not*1 of pGEX 4T

Nucleotide sequence of insert

GGATCCGAATTCGCCATGGAGCAGAAGCTTATCTCCGAGGAGGACCTCG
GTGGCGGCGAGAAGCAGAAGCACGACGGGCGGGTGAAGATCGGCCACTA
CATCCTGGGGGACACGCTGGGCGTCGGCACCTTCGGGAAAGTGAAGGTG
GGCAAGCACGAGTTGACTGGACATAAAGTTGCTGTGAAGATACTCAACC
GGCAGAAGATTCGAAGCCTGGACGTGGTCGGGAAAATCCGCAGAGAGAT
CCAGAACCTGAAGCTTTTCAGGCACCCTCATATAATCAAACTGTACCAG
GTCATCAGTACACCGTCTGATATTTTCATGGTCATGGAATATGTCTCAG
GAGGAGAGCTATTTGATTATATCTGTAAAAATGGAAGGTTGGACGAAAA
GGAGAGTCGACGTCTGTTCCAGCAGATCCTTTCTGGTGTGGACTATTGT
CACAGGCATATGGTGGTCCACAGAGATTTGAAACCTGAAAACGTCCTGC
TTGATGCACACATGAATGCAAAGATAGCCGACTTCGGTCTTTCAAACAT
GATGTCAGATGGTGAATTTTTAAGAGATAGCTGTGGCTCGCCCAATTAT
GCTGCACCAGAAGTAATTTCAGGAAGATTGTACGCAGGCCCTGAAGTAG
ACATCTGGAGCAGCGGGGTCATTCTCTATGCTTTGCTGTGTGGAACTCT
CCCTTTTGATGATGACCACGTGCCAACTCTTTTTAAGAAGATATGTGAC
GGGATATTTTATACCCCTCAGTATTTGAATCCCTCTGTAATAAGCCTTT
TGAAGCATATGCTGCAGGTAGATCCTATGAAGAGGGCCACAATAAAAGA
TATCAGGGAACATGAATGGTTTAAGCAGGACCTTCCAAAATATCTCTTT
CCTGAAGACCCGTCTTATAGTTCAACCATGATTGATGATGAAGCCTTAA
AAGAAGTGTGTGAGAAGTTCGAGTGCTCAGAGGAGGAGGTCCTCAGATC
CATCACACTGGCGGCCGC