Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active CAK1 [2 – 368]

Enzyme description:- CAK1 [2 – 368]

Clone Number:- DU 1089

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST and C-terminal His(6) tag

Purification method:- GSH Sepharose

Expression level:- <100 µg/L

Calculated molecular mass:- 69, 801 daltons

Purity:- 70 %

Activation protocol:- Constitutively active

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:-

Two step assay in which CAK1 activates CDK2 [DU 1043] / cyclin A2 [DU 1064].
The activity of CDK2 / cyclin A2 is then assayed against Histone H1 as a substrate (final concentration of 1 mg/ml) in the standard filter binding assay.

Assay buffer:-

50 mM Tris-HCl pH 7.5, 0. 1 mM EGTA, 0.1 % 2-mercaptoethanol,
0.1 mM sodium vanadate, 10 mM magnesium acetate

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Clone Data Sheet

CAK1 [3 – 368]

Protein CAK1 [3 – 368]

Clone number DU 1089

Species Yeast

Accession number U60192

Tags N-terminal GST + C-terminal His(6)

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA
VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**KLDSIDITHCQLVK
STRTARIYRSDTYAIKCLALDFDIPPHNAKFEVSILNKLGNKCKHILPL
LESKATDNNDLLLLFPFEEMNLYEFMQMHYKRDRRKKNPYYDLLNPSIP
IVADPPVQKYTNQLDVNRYSLSFFRQMVEGIAFLHENKIIHRDIKPQNI
MLTNNTSTVSPKLYIIDFGISYDMANNSQTSAEPMDSKVTDISTGIYKA
PEVLFGVKCYDGGVDVWSLLIIISQWFQRETSRMGHVPAMIDDGSDDMN
SDGSDFRLICSIFEKLGIPSIQKWEEVAQHGSVDAFVGMFGADGDGKYV
LDQEKDVQISIVERNMPRLDEIADVKVKQKFINCILGMVSFSPNERWSC
QRILQELEKP**GSHHHHHH

Native sequence Amino acids K2 – P368 (end) of yeast CAK1.
Residue K232 of fusion protein is equilivalent to K2 of the native enzyme. The GST tag is located at residues 1 - 220 and the His(6) tag is located at residues 601 – 606. The following sequence is present after the CAK1 sequence and before the His(6) tag, GS, residues 599 and 600.

Protease cleavage PreScission (LEVLFQGPL) at residues 221 – 229

Cloning sites *Bam*H1 and *Eco*R1 site of pGEX6P-1

Nucleotide sequence of insert

GGATCCAAACTGGATAGTATAGACATTACACACTGTCAGTTG
GTCAAATCTACTAGAACTGCTAGGATTTATAGGTCGGATACA
TATGCCATTAAATGTCTAGCACTAGATTTCGATATCCCGCCA
CATAACGCCAAATTCGAAGTATCGATATTAAACAAACTGGGC
AACAAATGTAAGCACATCTTACCTCTTCTAGAGTCTAAGGCT
ACCGATAATAATGACCTATTGTTGTTGTTTCCCTTTGAAGAG
ATGAACCTTTATGAGTTCATGCAAATGCACTATAAAAGAGAT
AGAAGAAAAAAAAAATCCCTATTACGATTTGCTAAATCCCAGT
ATCCCAATTGTTGCGGACCCCCCCGTTCAGAAATATACTAAT
CAATTGGACGTCAATCGGTATTCTTTGTCCTTTTTCCGGCAA
ATGGTTGAAGGGATTGCATTCTTACATGAGAACAAGATCATT
CACCGCGACATCAAACCGCAAAATATCATGCTAACAAACAAT
ACCAGCACCGTATCCCCAAAGTTGTACATAATTGATTTTGGC
ATCTCTTATGACATGGCAAATAACTCACAAACAAGTGCGGAA
CCCATGGATAGCAAGGTGACGGATATAAGCACAGGAATTTAC
AAGGCCCCAGAAGTGCTTTTTGGAGTAAAATGCTATGATGGT
GGCGTGGACGTGTGGTCGTTGTTGATAATTATTTCTCAGTGG
TTCCAGAGAGAAACAAGCCGTATGGGGCACGTTCCGGCCATG
ATTGATGACGGCAGCGACGACATGAACTCAGATGGAAGCGAT
TTCAGACTGATTTGCTCAATATTCGAAAAGTTGGGCATACCG
TCCATTCAGAAATGGGAAGAGGTTGCGCAACACGGCTCGGTT
GATGCATTTGTTGGTATGTTTGGTGCAGATGGCGATGGCAAG
TATGTACTGGACCAGGAGAAAGATGTACAGATTAGCATTGTT
GAGAGGAATATGCCTCGACTGGACGAGATTGCGGATGTCAAA
GTCAAGCAGAAGTTCATTAATTGTATCCTGGGGATGGTTTCA
TTTTCACCAAACGAAAGATGGAGCTGTCAAAGAATCTTGCAA
GAATTAGAAAAGCCAGGTTCACACCACCACCACCACCACtaa
gaattc