MRCPPU Reagents and Services

Standard Operating Procedure

Preparation of AAK1 [1 - 365]

Enzyme description:-	AAK1 [1 - 365]
Clone number:-	DU 61573
Source:-	Recombinant
Expression system:-	Baculovirus expression vector system
Tag:-	N-terminal GST
Purification method:-	GSH-Sepharose

Calculated molecular mass:-

Monoisotopic	67, 338.03 daltons
Average Mass	67, 381.63 daltons

[cysteines reduced, methionines have not been oxidised

Theoretical pI:-	6.39
Purity:-	>80 %

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, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Assay Buffer:-

50 mM Tris-HCl pH 7.5, 0.1mM EGTA, 10 mM DTT, 10 mM MgAc

Substrate:-

Myelin Basic protein Final concentration: 1 mg/ml

MRCPPU Reagents and Services

Clone Data Sheet

AAK1 [1 - 365]

Protein	AAK1 [1 - 365]
Clone number	DU 61573
Species	Human
Accession number	NM_014911.3
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MKKFFDSRREQGGSGLGSGSSGGGGSTSGLGSGYIGRVFGIGRQQVTVDEVLAEGGFAIVFLVRTSNGMKCALKRMFVNNEHDLQVCKREIQIMRDLSGHKNIVGYIDSSINNVSSGDVWEVLILMDFCRGGQVVNLMNQRLQTGFTENEVLQIFCDTCEAVARLHQCKTPIIHRDLKVENILLHDRGHYVLCDFGSATNKFQNPQTEGVNAVEDEIKKYTTLSYRAPEMVNLYSGKIITTKADIWALGCLLYKLCYFTLPFGESQVAICDGNFTIPDNSRYSQDMHCLIRYMLEPDPDKRPDIYQVSYFSFKLLKKECPIPNVQNSPIPAKLPEPVKASEAAAKKTQPKARLTDPIPTTETSIA**
Native sequence	Amino acids M1 – A365 (end residue is L961) of human AAK1. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission (LEVLFQGP) residues 221 – 228
Cloning sites	*Bam*H1 and *Not*1 sites of pFastBac-GST

Nucleotide Sequence Of Insert

```
ggatccATGAAGAAGTTTTTCGACTCCCGGCGAGAGCAGGGCGGCTCTG
GCCTGGGCTCCGGCTCCAGCGGAGGAGGGGGCAGCACCTCGGGCCTGGG
CAGTGGCTACATCGGAAGAGTCTTCGGCATCGGGCGACAGCAGGTCACA
GTGGACGAGGTGTTGGCGGAAGGTGGATTTGCTATTGTATTTCTGGTGA
GGACAAGCAATGGGATGAAATGTGCCTTGAAACGCATGTTTGTCAACAA
TGAGCATGATCTCCAGGTGTGCAAGAGAGAAATCCAGATAATGAGGGAT
CTTTCAGGACACAAGAATATTGTGGGTTACATTGATTCTAGTATCAACA
ACGTGAGTAGCGGTGATGTATGGGAAGTGCTCATTCTGATGGACTTTTG
TAGAGGTGGCCAGGTGGTAAACCTGATGAACCAGCGCCTGCAAACAGGC
TTTACAGAGAATGAAGTGCTCCAGATATTTTGTGATACCTGTGAAGCTG
TTGCCCGCCTGCATCAGTGCAAAACTCCTATTATCCACCGGGACCTGAA
GGTTGAAAACATCCTCTTGCATGACCGAGGCCACTATGTCCTGTGTGAC
TTTGGAAGCGCCACCAACAAATTCCAGAATCCACAAACTGAGGGAGTCA
ATGCAGTAGAAGATGAGATTAAGAAATACACAACGCTGTCCTATCGAGC
ACCAGAAATGGTCAACCTGTACAGTGGCAAAATCATCACTACGAAGGCA
GACATTTGGGCTCTTGGATGTTTGTTGTATAAATTATGCTACTTCACTT
TGCCATTTGGGGAAAGTCAGGTGGCAATTTGTGATGGAAACTTCACAAT
TCCTGATAATTCTCGATATTCTCAAGACATGCACTGCCTAATTAGGTAT
ATGTTGGAACCAGACCCTGACAAAAGGCCGGATATTTACCAGGTGTCCT
ACTTCTCATTTAAGCTACTCAAGAAAGAGTGCCCAATTCCAAATGTACA
GAACTCTCCCATTCCTGCAAAGCTTCCTGAACCAGTGAAAGCCAGTGAG
GCAGCTGCAAAAAAGACCCAGCCAAAGGCCAGACTGACAGATCCCATTC
CCACCACAGAGACTTCAATTGCAtgagcggccgc
```