Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active Choline Kinase Alpha [1 – 457]

Enzyme description:- CHKA [1 - 457]

Clone number:- DU 14189

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 5 mg/L

Calculated molecular mass:-
Monoisotopic 79, 022.13 daltons
Average Mass 79, 073.21 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.98

Purity:- >75 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-
12.5mM Glycine-NaOH (pH 8.5), 50mM KCl, 2.5mM MgCl2

Storage temperature:- -70 °C [Long term stability to be determined]

Assay:- ADP Glo

Assay buffer:-
25 mM glycine-NaOH pH 8.5, 67 mM KCl, 1mM DTT, 5 mM $MgCl_2$

Substrate:-
Choline Final concentration: 1 mM

Specific activity range:- To be determined

Clone Data Sheet

Choline Kinase Alpha [1 - 457]

Protein	CHKA [1 - 457]
Clone number	DU 14189
Species	Human
Accession number	NM_001277.2
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MKTKFCTGGEAEPS PLGLLLSCGSGSAAPAPGVGQQRDAASDLESKQLGGQQPPLALPPPPPL PLPLPLPQPPPPQPPADEQPEPRTRRRAYLWCKEFLPGAWRGLREDEFH ISVIRGGLSNMLFQCSLPDTTATLGDEPRKVLLRLYGAILQMRSCNKEG SEQAQKENEFQGAEAMVLESVMFAILAERSLGPKLYGIFPQGRLEQFIP SRRLDTEELSLPDISAEIAEKMATFHGMKMPFNKEPKWLFGTMEKYLKE VLRIKFTEESRIKKLHKLLSYNLPLELENLRSLLESTPSPVVFCHNDCQ EGNILLLEGRENSEKQKLMLIDFEYSSYNYRGFDIGNHFCEWMYDYSYE KYPFFRANIRKYPTKKQQLHFISSYLPAFQNDFENLSTEEKSIIKEEML LEVNRFALASHFLWGLWSIVQAKISSIEFGYMDYAQARFDAYFHQKRKL GV**
Native sequence	Amino acids M1 – V457 (end) of human Choline Kinase Alpha. 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*1site of pGEX 6P-1

Nucleotide Sequence of insert

agatctATGAAAACCAAATTCTGCACCGGGGGCGAGGCGGAGCCCTCGC
CGCTCGGGCTGCTGCTGAGCTGCGGTAGCGGCAGCGCGGCCCCGGCGCC
CGGCGTGGGGCAGCAGCGCGACGCCGCCAGCGACCTCGAGTCCAAGCAG
CTGGGCGGCCAACAGCCGCCGCTCGCGCTGCCCCCTCCGCCGCCGCTGC
CGCTGCCGCTGCCGCTGCCCCAGCCCCCGCCGCCGCAGCCGCCCGCAGA
CGAGCAGCCGGAGCCCCGGACGCGGCGCAGGGCCTATCTGTGGTGCAAG
GAGTTCCTGCCCGGCGCCTGGCGGGGCCTCCGCGAGGACGAGTTCCACA
TCAGTGTCATCAGAGGCGGCCTTAGCAACATGCTGTTCCAGTGCTCCCT
ACCTGACACCACAGCCACCCTTGGTGATGAGCCTCGGAAAGTGCTCCTG
CGGCTGTATGGAGCGATTTTGCAGATGAGGTCCTGTAATAAAGAGGGAT
CCGAACAAGCTCAGAAAGAAAATGAATTTCAAGGGGCTGAGGCCATGGT
TCTGGAGAGCGTTATGTTTGCCATTCTCGCAGAGAGGTCACTTGGGCCA
AAACTCTATGGCATCTTTCCCCAAGGCCGACTGGAGCAGTTCATCCCGA
GCCGGCGATTAGATACTGAAGAATTAAGTTTGCCAGATATTTCTGCAGA
AATCGCCGAGAAAATGGCTACATTTCATGGTATGAAAATGCCATTCAAT
AAGGAACCAAAATGGCTTTTTGGCACAATGGAAAAGTATCTAAAGGAAG
TGCTGAGAATTAAATTTACTGAGGAATCCAGAATTAAAAAGCTCCACAA
ATTGCTCAGTTACAATCTGCCCTTGGAACTGGAAAACCTGAGATCATTG
CTTGAATCTACTCCATCTCCAGTTGTATTTTGTCATAATGACTGTCAAG
AAGGTAATATCTTGTTGCTGGAAGGCCGAGAGAATTCTGAAAAACAGAA
ACTGATGCTCATTGATTTCGAATACAGCAGTTACAATTACAGGGGATTC
GACATTGGAAATCACTTCTGTGAGTGGATGTATGATTATAGCTATGAAA
AATACCCTTTTTTCAGAGCAAACATCCGGAAGTATCCCACCAAGAAACA
ACAGCTCCATTTTATTTCCAGTTACTTGCCTGCATTCCAAAATGACTTT
GAAAACCTCAGTACTGAAGAAAAATCCATTATAAAAGAAGAAATGTTGC
TTGAAGTTAATAGGTTTGCCCTTGCATCTCATTTCCTCTGGGGACTGTG
GTCCATTGTACAAGCCAAGATTTCATCTATTGAATTTGGGTACATGGAC
TACGCCCAAGCAAGGTTTGATGCCTATTTCCACCAGAAGAGGAAGCTTG
GGGTGtgagcggccgc