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:-
25 mM glycine-NaOH pH 8.5, 67 mM KCl, 2 mM EDTA, 270 mM Sucrose, 0.1 % B-mercaptoethanol 1 mM benzamidine, 0.2 mM PMSF

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

Assay:- ADP Glo

Assay buffer:-
12.5 mM glycine-NaOH pH 8.5, 50 mM KCl, 2.5 mM $MgCl_2$

Substrate:-
Choline chloride Final concentration: 0.2 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
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AATACCCTTTTTTCAGAGCAAACATCCGGAAGTATCCCACCAAGAAACA
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GAAAACCTCAGTACTGAAGAAAAATCCATTATAAAAGAAGAAATGTTGC
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GTCCATTGTACAAGCCAAGATTTCATCTATTGAATTTGGGTACATGGAC
TACGCCCAAGCAAGGTTTGATGCCTATTTCCACCAGAAGAGGAAGCTTG
GGGTGtgagcggccgc