Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active Choline Kinase Beta [1 – 395]

Enzyme description:- CHKB [1 - 395]

Clone number:- DU 19590

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 5 mg/L

Calculated molecular mass:-
Monoisotopic 74, 139.13 daltons
Average Mass 74, 186.83 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.48

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

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Clone Data Sheet

Choline Kinase Beta [1 - 395]

Protein	CHKB [1 - 395]
Clone number	DU 19590
Species	Human
Accession number	NM_005198
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MEPSPSEGSGAQPG** **LGPGRARAMAAEATAVAGSGAVGGCLAKDGLQQSKCPDTTPKRRRASSL** **SRDAERRAYQWCREYLGGAWRRVQPEELRVYPVSGGLSNLLFRCSLPDH** **LPSVGEEPREVLLRLYGAILQGVDSLVLESVMFAILAERSLGPQLYGVF** **PEGRLEQYIPSRPLKTQELREPVLSAAIATKMAQFHGMEMPFTKEPHWL** **FGTMERYLKQIQDLPPTGLPEMNLLEMYSLKDEMGNLRKLLESTPSPVV** **FCHNDIQEGNILLLSEPENADSLMLVDFEYSSYNYRGFDIGNHFCEWVY** **DYTHEEWPFYKARPTDYPTQEQQLHFIRHYLAEAKKGETLSQEEQRKLE** **EDLLVEVSRYALASHFFWGLWSILQASMSTIEFGYLDYAQSRFQFYFQQ** **KGQLTSVHSSS**
Native sequence	Amino acids M1 – 395 (end) of human Choline Kinase Beta. 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 site of pGEX 6P-1

Nucleotide Sequence of insert

ggatccATGGAACCGAGCCCGTCCGAAGGGAGCGGAGCGCAGCCTGGCC
TGGGGCCCGGTCGAGCCCGCGCCATGGCGGCCGAGGCGACAGCTGTGGC
CGGAAGCGGGGCTGTTGGCGGCTGCCTGGCCAAAGACGGCTTGCAGCAG
TCTAAGTGCCCGGACACTACCCCAAAACGGCGGCGCGCCTCGTCGCTGT
CGCGTGACGCCGAGCGCCGAGCCTACCAATGGTGCCGGGAGTACTTGGG
CGGGGCCTGGCGCCGAGTGCAGCCCGAGGAGCTGAGGGTTTACCCCGTG
AGCGGAGGCCTCAGCAACCTGCTCTTCCGCTGCTCGCTCCCGGACCACC
TGCCCAGCGTTGGCGAGGAGCCCCGGGAGGTGCTTCTGCGGCTGTACGG
AGCCATCTTGCAGGGCGTGGACTCCCTGGTGCTAGAAAGCGTGATGTTC
GCCATACTTGCGGAGCGGTCGCTGGGGCCCCAGCTGTACGGAGTCTTCC
CAGAGGGCCGGCTGGAACAGTACATCCCAAGTCGGCCATTGAAAACTCA
AGAGCTTCGAGAGCCAGTGTTGTCAGCAGCCATTGCCACGAAGATGGCG
CAATTTCATGGCATGGAGATGCCTTTCACCAAGGAGCCCCACTGGCTGT
TTGGGACCATGGAGCGGTACCTAAAACAGATCCAGGACCTGCCCCCAAC
TGGCCTCCCTGAGATGAACCTGCTGGAGATGTACAGCCTGAAGGATGAG
ATGGGCAACCTCAGGAAGTTACTAGAGTCTACCCCATCGCCAGTCGTCT
TCTGCCACAATGACATCCAGGAAGGGAACATCTTGCTGCTCTCAGAGCC
AGAAAATGCTGACAGCCTCATGCTGGTGGACTTCGAGTACAGCAGTTAT
AACTATAGGGGCTTTGACATTGGGAACCATTTTTGTGAGTGGGTTTATG
ATTATACTCACGAGGAATGGCCTTTCTACAAAGCAAGGCCCACAGACTA
CCCCACTCAAGAACAGCAGTTGCATTTTATTCGTCATTACCTGGCAGAG
GCAAAGAAAGGTGAGACCCTCTCCCAAGAGGAGCAGAGAAAACTGGAAG
AAGATTTGCTGGTAGAAGTCAGTCGGTATGCTCTGGCATCCCATTTCTT
CTGGGGTCTGTGGTCCATCCTCCAGGCATCCATGTCCACCATAGAATTT
GGTTACTTGGACTATGCCCAGTCTCGGTTCCAGTTCTACTTCCAGCAGA
AGGGGCAGCTGACCAGTGTCCACTCCTCATCCTGACTCCACCCTCCCAC
TCCTTGGATTTCTCCTGGAGCCTCCAGGGCAGGACCTTGGAGGGAGGAA
CAACGAGCAGAAGGCCCTGGCGACtgagcggccgc