

Standard Operating Procedure

Preparation of active CSK

<u>Enzyme description:-</u>	Active CSK
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	<i>E.coli</i>
<u>Expression conditions:-</u>	Induce expression with 100 μ M IPTG once culture reaches OD _{600nm} = 0.4. Express for 16 hours at 26°C.
<u>Tag:-</u>	N-terminal GST
<u>Purification method:-</u>	GSH- agarose.
<u>Expression level:-</u>	5mg/L
<u>Molecular mass:-</u>	77kDa by SDS-PAGE
<u>Purity:-</u>	>85%
<u>Contaminants:-</u>	No major contaminating proteins as judged by SDS-PAGE.
<u>Activation protocol:-</u>	None- constitutively active
<u>Enzyme storage buffer:-</u>	50 mM Tris/HCl pH 7.5, 50% glycerol, 150 mM NaCl, 0.1 mM EGTA, 0.1 % β -mercaptoethanol, 0.02% Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.
<u>Storage temperature:-</u>	-20°C

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CLONE DATA SHEET –human CSK

<u>Protein</u>	Human CSK C-src tyrosine kinase
<u>Accession number</u>	NM_004383
<u>Tags</u>	GST
<u>Bacterially-expressed protein</u>	MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDE GDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMARIY IADKHNMLGGCPKERAIEISMLEGAVLDIRYGVSRAY SKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRI EAIPQIDKYLKSSKYIAWPLQGQWQATFGGGDHPKPS DLVPRGSMASAIQAAWPSGTECIAKYNFHGTAEQDLP FCKGDVLTIVAVTKDPNWKAKNKGREGIIPANYV QKREGVKAGTKLSLMPWFHKGITREQAERLLYPPET GLFLVRESTNYPGDYTLCSVCDGKVEHYRIMYHASKL SIDEVYFENLMQLVEHYTSDADGLCTRLIKPKVMEG TVAAQDEFYRSGWALNMKELKLLQTIGKGEFGDVM LGDYRGNKVAVKCIKNATAQAFLAEASVMTQLRH SNLVQLLGVIVEEKGGLYIVTEYMAKGSVDYLRSG RSVLGGDCLLKFSLDVCEAMEYLEGNFVHRDLAAR NVLVSEDNVAKVSDFGTLKEASSTQDTGKLPVKWTA PEALREKKFSTKSDVWSFGILLWEIYSFGRVPYPRIPK DVVPRVEKGYKMDAPDGCPPAVYEVMMKNCWHLDA AMRPSFLQLREQLLEHIKTHELHL*
<u>Native sequence</u>	Protein Sequence of expressed construct. 1M of CSK is 227M of expressed protein. Thrombin site (LVPRGS) residues 221 –226.
<u>Protease cleavage site</u>	Thrombin site (LVPRGS) residues 221 –226.
<u>Cloning sites</u>	BamH1 site of pGEX4T-1

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Nucleotide

Sequence of CSK

ORF

ATGTCAGCAATACAGGCCGCCTGGCCATCCGGTACAGAATG
TATTGCCAAGTACAACCTCCACGGCACTGCCGAGCAGGACC
TGCCCTTCTGCAAAGGAGACGTGCTCACCATTGTGGCCGTC
ACCAAGGACCCCAACTGGTACAAAGCCAAAAACAAGGTGGG
CCGTGAGGGCATCATCCCAGCCAACTACGTCCAGAAGCGGG
AGGGCGTGAAGGCGGGTACCAAACCTCAGCCTCATGCCTTGG
TTCCACGGCAAGATCACACGGGAGCAGGCTGAGCGGCTTCT
GTACCCGCCGGAGACAGGCCTGTTCTTGGTGCGGGAGAGCA
CCAACTACCCCGGAGACTACACGCTGTGCGTGAGCTGCGAC
GGCAAGGTGGAGCACTACCGCATCATGTACCATGCCAGCAA
GCTCAGCATCGACGAGGAGGTGTACTTTGAGAACCTCATGC
AGCTGGTGGAGCACTACACCTCAGACGCAGATGGACTCTGT
ACGCGCCTCATTAACCAAAGGTCATGGAGGGCACAGTGGC
GGCCCAGGATGAGTTCTACCGCAGCGGCTGGGCCCTGAACA
TGAAGGAGCTGAAGCTGCTGCAGACCATCGGGAAGGGGGAG
TTCGGAGACGTGATGCTGGGCGATTACCGAGGGAACAAAGT
CGCCGTCAAGTGCATTAAGAACGACGCCACTGCCCAGGCCT
TCCTGGCTGAAGCCTCAGTCATGACGCAACTGCGGCATAGC
AACCTGGTGCAGCTCCTGGGCGTGATCGTGGAGGAGAAGGG
CGGGCTCTACATCGTCACTGAGTACATGGCCAAGGGGAGCC
TTGTGGACTACCTGCGGTCTAGGGGTCGGTCAGTGCTGGGC
GGAGACTGTCTCCTCAAGTTCTCGCTAGATGTCTGCGAGGC
CATGGAATACCTGGAGGGCAACAATTTTCGTGCATCGAGACC
TGGCTGCCCCGCAATGTGCTGGTGTCTGAGGACAACGTGGCC
AAGGTCAGCGACTTTGGTCTCACCAGGAGGCGTCCAGCAC
CCAGGACACGGGCAAGCTGCCAGTCAAGTGGACAGCCCCTG
AGGCCCTGAGAGAGAAGAAATTCTCCACTAAGTCTGACGTG
TGGAGTTTCGGAATCCTTCTCTGGGAAATCTACTCCTTTGG
GCGAGTGCCTTATCCAAGAATTCCCCTGAAGGACGTGCTCC
CTCGGGTGGAGAAGGGCTACAAGATGGATGCCCCGACGGC
TGCCCGCCCGCAGTCTATGAAGTCATGAAGAACTGCTGGCA
CCTGGACGCCGCCATGCGGCCCTCCTTCCTACAGCTCCGAG
AGCAGCTTGAGCACATCAAAACCCACGAGCTGCACCTGTGA