

Certificate of Analysis

CSK, active

(Recombinant enzyme expressed in *E. coli* cells)

Item # 14-458, 14-458-K, 14-458M

Parent Lot # D8CN060U

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: Recombinant full-length human CSK, containing an N-terminal GST-tag incorporating a PreScission Protease cleavage site. Expressed in *E. coli* cells. Purified using GST agarose. Purity 100%. MW = 77.6kDa.

Formulation: 2.742mg/ml of enzyme in 50mM Tris/HCl, pH7.5, 0.1mM EGTA, 150mM NaCl, 1mM benzamidine, 0.03% Brij-35, 0.2mM PMSF, 0.1% 2-mercaptoethanol, 50% glycerol. Liquid at -20°C

Specific Activity (Parent lot# D8CN060U): 357U/mg where one unit equals the incorporation of 1nmol of phosphate per min into 0.1mg/ml poly(Glu,Tyr) (4:1), at 30°C using 100µM ATP.

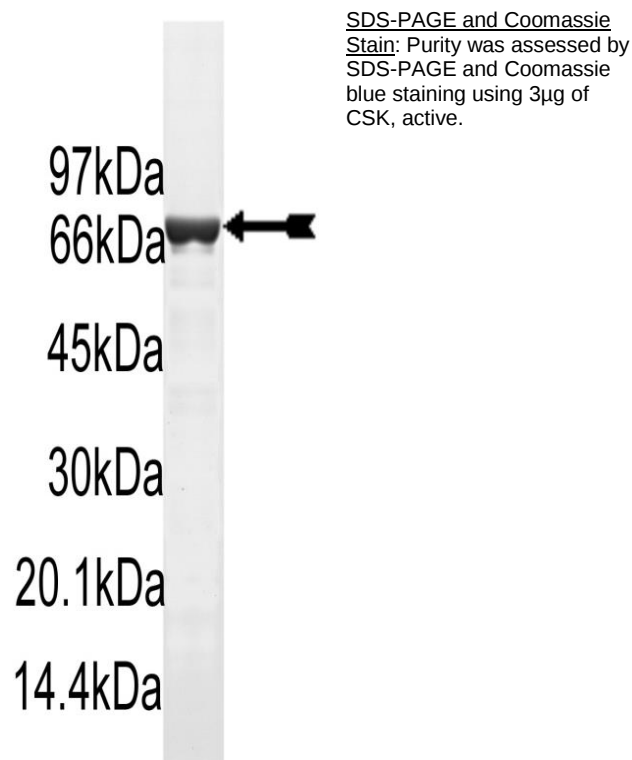
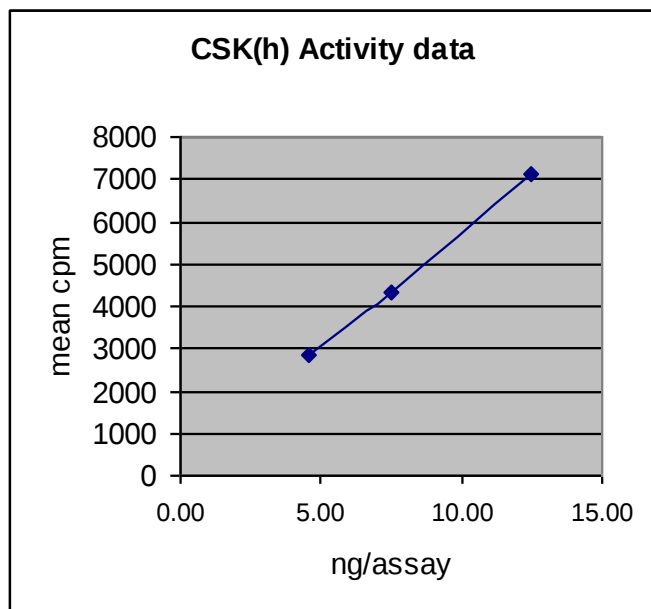
Storage and Stability: On receipt of material store at -20°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 4.5–12.5ng of this lot of enzyme phosphorylated 0.1mg/ml poly(Glu,Tyr) (4:1) in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.

MS Tryptic Fingerprint: Confirmed identity as CSK with the translated native sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

1. **10 x Reaction Buffer:** 500mM Tris/HCl pH7.5, 1mM EGTA, 1% 2-mercaptoethanol, 1mM Na₃VO₄.
2. **Poly(Glu,Tyr) (4:1):** use a final assay concentration of 0.1mg/ml. Make up a 1mg/ml stock. Use 2.5µl of stock per assay point.
3. **MnCl₂:** Use at a final concentration of 5mM. Make up a 50mM stock. Use 2.5µl of stock per assay point.
4. **CSK, active:** Dilute with 50mM Tris/HCl pH7.5, 0.1mM EGTA, 0.1% 2-mercaptoethanol, 0.1mM Na₃VO₄, 1mg/ml BSA. Use 4.5–12.5ng per assay point.
5. **[γ-³³P]ATP:** 2.5 x magnesium acetate/[γ-³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 2.5µl of 10 x reaction buffer per assay to wells.
2. Add 2.5µl of **poly(Glu,Tyr) (4:1)**.
3. Add 2.5µl **(4.5–12.5ng) CSK, active**.
4. Add 2.5µl of MnCl₂
5. Add 5µl of dH₂O.
6. Add 10µl of diluted [γ-³³P]ATP mixture.
7. Incubate for 10 minutes at 30°C.
8. Stop the reaction by adding 5µl of 3% phosphoric acid.
9. Transfer a 10µl aliquot onto the appropriate area of a **Filtermat A**.
10. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
11. Wash the filtermat once for 2 minutes with methanol.
12. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
13. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1µl of 30% phosphoric acid.

CSK Sequence Information

Protein	human CSK
Tags	N-terminal GST incorporating PreScission Protease cleavage site
Native sequence	M232 of the recombinant protein is equivalent to M1 of human CSK
Accession number	GenBank X60114 (CDS GB 30314)

Recombinant CSK amino acid sequence:

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1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAIQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LEVLFQGPLG SMSAIQAAMP
241 SGTECIAKYN FHGTAEQDLP FCKGDLTIV AVTKDPNWKY AKNKVGREGI IPANYVQKRE
301 GVKAGTKLSL MPWFHGKTR EQAERLLYPP ETGLFLVRES TNYPGDYTLT VSCDGKVEHY
361 RIMYHASKLS IDEEVYFENL MQLVEHYTSD ADGLCTRLIK PKVMEGTVAQ QDEFYRSGWA
421 LNMKELKLLQ TIGKGEFGDV MLGDYRGNKV AVKCIKNDAT AQAFLAEASV MTQLRHSNLV
481 QLLGVIVEEK GGLYIVTEYM AKGSLVDYLR SRGRSVLGGD CLLKFSLDVC EAMEYLEGNN
541 FVHRDLAARN VLVSEDNVAK VSDFGLTKEA SSTQDTGKLP VKWTAPEALR EKKFSTKSDV
601 WSGILLWEI YSFGRPVYPR IPLKDVVPRV EKGKMDAPD GCPPAVYEVN KNCWHLDAAM
661 RPSFLQLREQ LEHIKTHELH L

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Recombinant CSK nucleotide sequence:

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2041 ctgtga
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