

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of CISD1 [1 – 108 mouse]

Enzyme description:- CISD1 [1 – 108 mouse]

Clone number:- DU 51315

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 38, 895.79 daltons

Average Mass 38, 921.12 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 7.10

Purity:- >80 %

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70

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Clone Data Sheet

CISD1 [1 – 108 mouse]

<u>Protein</u>	CISD1 [1 – 108 mouse]
<u>Clone number</u>	DU 51315
<u>Species</u>	Mouse
<u>Accession number</u>	Q91WS0
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKL TQSM A I RY IADKHNMLGGCPKERA E I S M L E GAVLDIRYGVSRIAYS KDFETLKVDFLSKLP E M L K M F E D R L C H K T Y L N GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAI PQIDKY LKSSKYIAWPLQGWQATFGGGDHPKSD LEVL F Q G P L G S M G L S S N S A V RVEWIAAVTFAAGTAALGYLAYKKFYAKENRTKAMVNLQIQKDNPKVV HAFDMEDLGDKAVYCRCWRSKKFPFCDGAHIKHNEETGDNVGPLI IKK KET</p>
<u>Native sequence</u>	<p>Amino acids M1 – T108 (end) of mouse CISD1. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.</p>
<u>Protease cleavage</u>	PreScission (LEVL F Q G P) residues 221 – 228.
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> 1 site of pGEX6P-1

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**Nucleotide
sequence of
insert**

gggatccATGGGCCTCAGCTCCAACCTCCGCTGTGCGAGTTGAGTGGAT
CGCGGCCGTCACCTTTGCTGCTGGCACAGCCGCTCTCGGTTACCTGGC
TTACAAGAAGTTCTACGCTAAAGAGAATCGCACCAAAGCTATGGTGAA
TCTTCAGATCCAGAAAGACAACCCGAAGGTGGTGCATGCCTTCGACAT
GGAGGATCTGGGGGATAAGGCCGTGTACTGCCGATGCTGGAGGTCTAA
AAAGTTCCCCTTCTGCGATGGGGCTCACATAAAGCACAAACGAAGAGAC
TGGCGACAACGTAGGACCTCTGATCATCAAGAAAAAGGAAACCTaagc
ggccgc