

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of CISD1 [1 - 108]

Enzyme description:- CISD1 [1 – 108]

Clone number:- DU 44399

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 39, 792.20 daltons

Average Mass 39, 818.08 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.83

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]

Protein CISD1 [1 – 108]

Clone number DU 44399

Species Human

Accession number NM_018464.4

Tags N-terminal GST

Bacterially expressed protein MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTSMAIIRYIADKHNMLGGCPKERAETSMLE
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY
LKSSKYIAWPLQGWQATFGGGDHPKSDLEVLFOGPLGSENLVYFQMSL
TSSSSVRVEWIAAVTIAAGTAAIGYLAYKRFYVKDHRNKAMINLHIQK
DNPKIVHAFDMEDLGDKAVYCRWRSKKFPFCGHAHTKHNEETGDNVG
PLI I K K K E T

Native sequence Amino acids M1 – T108 (end) of human CISD1.
Residue M238 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.

Protease cleavage PreScission (LEVLFOGQP) residues 221 – 228 and rTEV (ENLYFQ) residues 232 – 237.

Cloning sites *Bam*H1 and *Not*1 site of pGEX6P-1

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**Nucleotide
sequence of
insert**

ggatccgaaaacctgtattttcagATGAGTCTGACTTCCAGTTCCAGC
GTACGAGTTGAATGGATCGCAGCAGTTACCATTGCTGCTGGGACAGCT
GCAATTGGTTATCTAGCTTACAAAAGATTTTATGTTAAAGATCATCGA
AATAAAGCTATGATAAACCTTCACATCCAGAAAGACAACCCCAAGATA
GTACATGCTTTTGACATGGAGGATTTGGGAGATAAAGCTGTGTACTGC
CGTTGTTGGAGGTCCAAAAGTTCCATTCTGTGATGGGGCTCACACA
AAACATAACGAAGAGACTGGAGACAATGTGGGCCCTCTGATCATCAAG
AAAAAAGAACTtaagcggccgc