Standard Operating Procedure

Preparation of Calcium Regulated Heat Stable Protein 1 24 [2 – 147]

Enzyme description:- CRHSP 24 [2 - 147]

Clone number:- DU 743

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST and HA

Purification method:- GSH Sepharose

Expression level:- 2 mg/L

Calculated molecular mass:-
Monoisotopic   44, 317.37 daltons
Average Mass   44, 345.96 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.08

Purity:- >80 %

Activation protocol:- Constitutively active

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, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -80 °C

Assay:- Substrate for PKB and RSK

Division of Signal Tranduction Therapy

Clone Data Sheet

CRHSP 24 [2 – 147]

Protein	CRHSP 24 [2 – 147]
Clone number	DU 743
Species	Human
Accession number	NM_014316
Tags	N-terminal GST and HA [YPYDVPDYA]
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSD<u>LEVLFQGPL</u>GSPEFATMYPYDVPDY A**SSEPPPPPQPPTHQASVGLLDTPRSRERSPSPLRGNVVPSPLPTRRTR TFSATVRASQGPVYKGVCKCFCRSKGHGFITPADGGPDIFLHISDVEGE YVPVEGDEVTYKMCSIPPKNEKLQAVEVVITHLAPGTKHETWSGHVISS**
Native sequence	Amino acids S2 – S147 (end) of human CRHSP 24. Residue S247 of the fusion protein is equivalent to E2 of the native enzyme. The GST tag is located at residues 1 – 220 and the HA tag is located at residues 238 – 246.
Protease cleavage	PreScission (<u>LEVLFQGPL</u>) residues 221 - 229
Cloning sites	*Eco*R1 and *Sal*1site of pGEX 6P-1

Nucleotide
Sequence of insert

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gaattcgccaccatgtacccatacgatgtgccagattacgccTCATCTG
AGCCTCCCCCACCACCACAGCCCCCCACCCATCAAGCTTCAGTCGGGCT
GCTGGACACCCCTCGGAGCCGTGAGCGCTCACCATCCCCTCTGCGGGGC
AACGTGGTCCCAAGCCCACTGCCCACTCGCCGGACGAGGACCTTCTCGG
CGACGGTGCGGGCTTCACAGGTCCCCGTCTACAAAGGAGTCTGCAAATG
CTTCTGCCGGTCCAAGGGCCATGGCTTCATTACCCCAGCTGATGGCGGC
CCCGACATCTTCCTGCACATCTCTGATGTGGAAGGGGAGTATGTCCCAG
TGGAAGGCGACGAGGTCACCTATAAAATGTGCTCCATCCCACCCAAGAA
TGAGAAGCTGCAGGCCGTGGAGGTCGTCATCACTCACCTGGCACCAGGC
ACCAAGCATGAGACCTGGTCTGGACATGTCATCAGCTCCtaggtcgac
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