

*Division of Signal Transduction Therapy*

**Standard Operating Procedure**

**Preparation of CREB [1 - 283]**

**Protein description:-** CREB [1 - 283]

**Clone number:-** DU 1570

**Source:-** Recombinant

**Expression system:-** *E.coli*

**Tag:-** N-terminal GST

**Purification method:-** GSH Sepharose

**Expression level:-** 1 mg/L

**Calculated molecular mass:-** 56, 100 daltons

**Purity:-** 75 %

**Enzyme storage buffer:-**

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

**Storage temperature:-** -20 °C

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**CLONE DATA SHEET**

**CREB [1 – 282]**

<b><u>Protein</u></b>	CREB [1 – 282]
<b><u>Clone number</u></b>	DU 1570
<b><u>Species</u></b>	Rat
<b><u>Accession no</u></b>	NM_031017
<b><u>Tags</u></b>	N-terminal GST
<b><u>Expressed protein</u></b>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKF ELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEI SMLEGAVLDIRYGVSR IAYSKDFETLKVDFLSKLPEMLKMFEDRLC HKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIE AIPQIDKYLKSSKYIAWPLQGQATFGGGDHPPKSDLVPRGSPGMT <b>MESGADNQQSGDAAVTEAESQQMTVQAQPQIATLAQVSM PAAHATS</b> <b>SAPTVTTLVQLPNGQTVQVHGVIQAAQPSVIQSPQVQTVQSSCKDLK</b> <b>RLFSGTQISTIAESEDSESVDSVTDSQKRREILSRRPSYRKILND</b> <b>LSSDAPGVPRIEEKSEEETSAPAITTVTVPTPIYQTSSGQYIAIT</b> <b>QGGAIQLANNGTDGVQGLQTLTMTNAAATQPGTTILQYAQT TDGQQ</b> <b>ILVPSNQVVVQAASGDVQTYQIRTAPTSTIAPGVVMASSPALPTQP</b> <b>AEEAAV</b></p>
<b><u>Native sequence</u></b>	<p>Amino acids M1 – A283 of rat CREB. [Full length protein ends at residue D341] Residue M229 of the fusion protein is equivalent to M1 of the native protein. The GST tag is located at residues 1 – 220. The following amino acid is present after the CREB sequence, V, at residue 512.</p> <p>The following amino acid substitution is present: D – E, where D4 of the native sequence is <a href="#">E232</a> of the fusion protein</p>
<b><u>Protease cleavage</u></b>	Thrombin ( <u>LVPRGS</u> ) residues 221 - 226
<b><u>Cloning sites</u></b>	<i>Sma</i> 1 and <i>Xba</i> 1 of pGEX 2T

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**Nucleotide  
sequence of insert**

GGATCCCCGGGAATGACCATGGAATCTGGAGCAGACAACCAGCAGA  
GTGGAGATGCTGCTGTAACAGAAGCTGAAAGTCAACAAATGACAGT  
TCAAGCCCAGCCACAGATTGCCACATTAGCCCAGGTATCCATGCCA  
GCAGCTCATGCGACGTCATCTGCTCCCAGTGTAAACCTTAGTGCAGC  
TGCCCAATGGGCAGACAGTCCAGGTCCATGGGGTCATCCAGGCGGC  
CCAGCCATCAGTTATTCAGTCTCCACAAGTCCAAACAGTTTCAGTCT  
TCCTGTAAGGACTTAAAAAGACTTTTCTCCGGAACCTCAGATTTCAA  
CTATTGCAGAAAGTGAAGATTCACAGGAGTCTGTGGATAGTGTAAC  
TGATTCCCAAAAACGAAGGGAAATCCTTTCAAGGAGGCCTTCCTAC  
AGGAAAATTTTGAATGACTTATCTTCTGATGCACCAGGGGTGCCAA  
GGATTGAAGAAGAAAAATCAGAAGAAGAGACTTCAGCCCCTGCCAT  
CACCACTGTAACAGTGCCAACCCCGATTTACCAAACCTAGCAGTGGG  
CAGTATATTGCCATTACCCAGGGAGGAGCAATACAGCTGGCTAACA  
ATGGTACCGATGGGGTACAGGGCCTGCAGACATTAACCATGACCAA  
TGCAGCTGCCACTCAGCCGGGTACTACCATTCTACAATATGCACAG  
ACCACTGATGGACAGCAGATTCTAGTGCCCAGCAACCAAGTTGTTG  
TTCAAGCTGCCTCTGGTGTGTACAAACATAACCAGATTCGCACAGC  
ACCACTAGCACCATTGCCCTGGAGTTGTTATGGCGTCCTCCCA  
GCACTTCCTACACAGCCTGCTGAAGAAGCAGCAGTctag