Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of CREB [1 – 341]

Enzyme description:- CREB [1 - 341]

Clone number:- DU 35579

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 64, 450.75 daltons
Average Mass 64, 491.36 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.67

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, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Division of Signal Tranduction Therapy

Clone Data Sheet

CREB [1 - 341]

Protein	CREB [1 - 341]
Clone number	DU 35579
Species	Human
Accession number	NM_134442.3
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPGIPGSTRAAA**MTM** **ESGAENQQSGDAAVTEAENQQMTVQAQPQIATLAQVSMPAAHATSSAPT** **VTLVQLPNGQTVQVHGVIQAAQPSVIQSPQVQTVQSSCKDLKRLFSGTQ** **ISTIAESEDSQESVDSVTDSQKRREILSRRPSYRKILNDLSSDAPGVPR** **IEEEKSEEETSAPAITTVTVPTPIYQTSSGQYIAITQGGAIQLANNGTD** **GVQGLQTLTMTNAAATQPGTTILQYAQTTDGQQILVPSNQVVVQAASGD** **VQTYQIRTAPTSTIAPGVVMASSPALPTQPAEEAARKREVRLMKNREAA** **RECRRKKKEYVKCLENRVAVLENQNKTLIEELKALKDLYCHKSD**
Native sequence	Amino acids M1 – D341 (end) of human CREB. Residue M243 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission (LEVLFQGP) residues 221 – 228
Cloning sites	*Not*1 sites of pGEX6P-2

<u>Nucleotide</u> <u>Sequence of insert</u>

```
gcggccgcaATGACCATGGAATCTGGAGCCGAGAACCAGCAGAGTGGAGATGCAGCTGTAACAGAA
GCTGAAAACCAACAAATGACAGTTCAAGCCCAGCCACAGATTGCCACATTAGCCCAGGTATCTATG
CCAGCAGCTCATGCAACATCATCTGCTCCCACCGTAACTCTAGTACAGCTGCCCAATGGGCAGACA
GTTCAAGTCCATGGAGTCATTCAGGCGGCCCAGCCATCAGTTATTCAGTCTCCACAAGTCCAAACA
GTTCAGTCTTCCTGTAAGGACTTAAAAAGACTTTTCTCCGGAACACAGATTTCAACTATTGCAGAA
AGTGAAGATTCACAGGAGTCAGTGGATAGTGTAACTGATTCCCAAAAGCGAAGGGAAATTCTTTCA
AGGAGGCCTTCCTACAGGAAAATTTTGAATGACTTATCTTCTGATGCACCAGGAGTGCCAAGGATT
GAAGAAGAGAAGTCTGAAGAGGAGACTTCAGCACCTGCCATCACCACTGTAACGGTGCCAACTCCA
ATTTACCAAACTAGCAGTGGACAGTATATTGCCATTACCCAGGGAGGAGCAATACAGCTGGCTAAC
AATGGTACCGATGGGGTACAGGGCCTGCAAACATTAACCATGACCAATGCAGCAGCCACTCAGCCG
GGTACTACCATTCTACAGTATGCACAGACCACTGATGGACAGCAGATCTTAGTGCCCAGCAACCAA
GTTGTTGTTCAAGCTGCCTCTGGAGACGTACAAACATACCAGATTCGCACAGCACCCACTAGCACT
ATTGCCCCTGGAGTTGTTATGGCATCCTCCCCAGCACTTCCTACACAGCCTGCTGAAGAAGCAGCA
CGAAAGAGAGAGGTCCGTCTAATGAAGAACAGGGAAGCAGCTCGAGAGTGTCGTAGAAAGAAGAAA
GAATATGTGAAATGTTTAGAAAACAGAGTGGCAGTGCTTGAAAATCAAAACAAGACATTGATTGAG
GAGCTAAAAGCACTTAAGGACCTTTACTGCCACAAATCAGATtaagcggccgc
```