Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active OSR1 T185E [1 – 527]

Enzyme description:- OSR1 T185E [1 - 527]

Clone number:- DU 6231

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 84, 774.66 daltons
Average Mass 84, 828.30 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.86

Purity:- 85 %

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:- -70 °C

Assay buffer:-
50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1 % 2–mercaptoethanol, 10 mM MgAc

Substrate:-
CATCHtide [RRHYYYDTHTNTYYLRTFGHNTRR]
Final concentration: 300 μM

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Clone Data Sheet

OSR1 T185E [1 - 527]

Protein OSR1 T185E [1 - 527]

Clone number DU 6231

Species Human

Accession number NM_005109.2

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA
VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MSEDSSALPWSINR
DDYELQEVIGSGATAVVQAAYCAPKKEKVAIKRINLEKCQTSMDELLKE
IQAMSQCHHPNIVSYYTSFVVKDELWLVMKLLSGGSVLDIIKHIVAKGE
HKSGVLDESTIATILREVLEGLEYLHKNGQIHRDVKAGNILLGEDGSVQ
IADFGVSAFLATGGDITRNKVRKEFVGTPCWMAPEVMEQVRGYDFKADI
WSFGITAIELATGAAPYHKYPPMKVLMLTLQNDPPSLETGVQDKEMLKK
YGKSFRKMISLCLQKDPEKRPTAAELLRHKFFQKAKNKEFLQEKTLQRA
PTISERAKKVRRVPGSSGRLHKTEDGGWEWSDDEFDEESEEGKAAISQL
RSPRVKESISNSELFPTTDPVGTLLQVPEQISAHLPQPAGQIATQPTQV
SLPPTAEPAKTAQALSSGSGSQETKIPISLVLRLRNSKKELNDIRFEFT
PGRDTAEGVSQELISAGLVDGRDLVIVAANLQKIVEEPQSNRSVTFKLA
SGVEGSDIPDDGKLIGFAQLSIS**

Native sequence Amino acids M1 – S527 (end) of human OSR1.
Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
The enzyme has a T185E mutation in order to mimic phosphorylation of the enzyme. Residue T185 is equivalent to E416 of the fusion protein.

Protease cleavage PreScission (LEVLFQGP) residues 221 - 229

Cloning sites *Bam*H1 and *Not*1 sites of pGEX 6P-1

Nucleotide Sequence of insert

ggatccATGTCCGAAGACTCGAGCGCCCTGCCCTGGTCCATCAACAGGG
ACGATTACGAGCTGCAGGAGGTGATCGGGAGTGGAGCAACTGCTGTAGT
CCAAGCAGCTTATTGTGCCCCTAAAAAGGAGAAAGTGGCAATCAAACGG
ATAAACCTTGAGAAATGTCAAACTAGCATGGATGAACTCCTGAAAGAAA
TTCAAGCCATGAGTCAATGCCATCATCCTAATATTGTATCTTACTACAC
ATCTTTTGTGGTAAAAGATGAGCTGTGGCTTGTCATGAAGCTGCTAAGT
GGAGGTTCTGTTCTGGATATTATTAAGCACATTGTGGCAAAAGGGGAAC
ACAAAAGTGGAGTCCTAGATGAATCTACCATTGCTACGATACTCCGAGA
AGTACTGGAAGGGCTGGAATATCTGCATAAAAATGGACAGATCCACAGA
GATGTGAAAGCTGGAAACATTCTTCTTGGAGAAGATGGCTCAGTACAGA
TTGCAGACTTTGGGGTTAGTGCTTTTTTAGCAACTGGTGGTGATATTAC
CCGAAATAAAGTGAGAAAGGAGTTTGTTGGCACCCCTTGTTGGATGGCA
CCTGAAGTTATGGAACAGGTCCGTGGTTATGATTTCAAAGCTGATATTT
GGAGTTTTGGAATTACAGCAATTGAATTGGCTACAGGGGCGGCTCCTTA
TCATAAATATCCACCAATGAAGGTTTTAATGCTGACACTGCAGAACGAT
CCTCCTTCTTTGGAAACTGGTGTTCAAGATAAAGAAATGCTGAAAAAAT
ATGGAAAATCATTTAGAAAAATGATTTCATTGTGCCTTCAAAAAGATCC
AGAAAAAAGACCAACAGCAGCAGAACTATTAAGGCACAAATTTTTCCAG
AAAGCAAAGAATAAAGAATTTCTTCAAGAAAAAACATTGCAGAGAGCAC
CAACCATTTCTGAAAGAGCAAAAAAGGTTCGGAGAGTACCAGGTTCCAG
TGGGCGTCTTCATAAGACAGAGGATGGAGGCTGGGAGTGGAGTGATGAT
GAATTTGATGAAGAAAGTGAGGAAGGGAAAGCAGCAATTTCACAACTCA
GGTCTCCCCGAGTGAAAGAATCAATATCAAATTCTGAGCTCTTTCCAAC
AACTGATCCTGTGGGTACTTTGCTCCAAGTTCCAGAACAGATCTCTGCT
CATCTACCTCAGCCAGCTGGGCAGATTGCTACACAGCCAACTCAAGTCT
CTCTCCCACCCACCGCAGAGCCAGCAAAAACAGCTCAGGCTTTGTCTTC
AGGATCAGGTTCACAAGAAACCAAGATCCCAATCAGTCTAGTACTAAGA
TTAAGGAATTCCAAAAAAGAACTAAATGATATTCGATTTGAATTTACTC
CTGGGAGAGATACAGCAGAGGGTGTCTCTCAGGAACTCATTTCTGCTGG
CCTGGTCGACGGAAGGGATTTAGTAATAGTGGCAGCTAATTTGCAGAAA
ATTGTGGAAGAACCTCAGTCAAATCGATCTGTCACTTTCAAACTGGCAT
CTGGTGTCGAAGGCTCAGATATTCCTGATGATGGTAAACTGATAGGATT
TGCCCAGCTCAGCATCAGCtaagcggccgc