Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active OSR1 T185E [1 – 342]

Enzyme description:- OSR1 T185E [1 - 342]

Clone number:- DU 6363

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 65, 178.42 daltons
Average Mass 65, 220.34 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.46

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 - 342]

Protein	OSR1 T185E [1 - 342]
Clone number	DU 6363
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** **PTISERAKKVRRVPGSSGRLHKTEDGGWEWSDDE**
Native sequence	Amino acids M1 – E342 of human OSR1. [Full length protein ends at residue S527] 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
CAACCATTTCTGAAAGAGCAAAAAAGGTTCGGAGGGTACCAGGTTCCAG
TGGGCGTCTTCATAAGACAGAGGATGGAGGCTGGGAGTGGAGTGATGAT
GAATAGGATGAAGAAAGTGAGGAAGGGAAAGCAGCAATTTCACAACTCA
GGTCTCCCCGAGTGAAAGAATCAATATCAAATTCTGAGCTCTTTCCAAC
AACTGATCCTGTGGGTACTTTGCTCCAAGTTCCAGAACAGATCTCTGCT
CATCTACCTCAGCCAGCTGGGCAGATTGCTACACAGCCAACTCAAGTCT
CTCTCCCACCCACCGCAGAGCCAGCAAAAACAGCTCAGGCTTTGTCTTC
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TTAAGGAATTCCAAAAAAGAACTAAATGATATTCGATTTGAATTTACTC
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CCTGGTCGACGGAAGGGATTTAGTAATAGTGGCAGCTAATTTGCAGAAA
ATTGTGGAAGAACCTCAGTCAAATCGATCTGTCACTTTCAAACTGGCGT
CTGGTGTCGAAGGCTCAGATATTCCTGATGATGGTAAACTGATAGGATT
TGCCCAGCTCAGCATCAGCtaagcggccgc