Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of OSR1 D164A [1 - 477]

Enzyme description:- OSR1 D164A [1 – 477]

Clone number:- DU 2961

Source:- Recombinant

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

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 86, 135.28 daltons
Average Mass 86, 189.88 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.86

Purity:- >80 %

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 150 mM NaCl, 50 % glycerol, 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

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Clone Data Sheet

OSR1 D164A [1 - 527]

Protein	OSR1 D164A [1 - 527]
Clone number	DU 2961
Species	Human
Accession number	NM_005109.2
Tags	N-terminal GST + HA
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSATM*YPYDVP* *DYA***MSEDSSALPWSINRDDYELQEVIGSGATAVVQAAYCAPKKEKVAI** **KRINLEKCQTSMDELLKEIQAMSQCHHPNIVSYYTSFVVKDELWLVMK** **LLSGGSVLDIIKHIVAKGEHKSGVLDESTIATILREVLEGLEYLHKNG** **QIHRDVKAGNILLGEDGSVQIAAFGVSAFLATGGDITRNKVRKTFVGT** **PCWMAPEVMEQVRGYDFKADIWSFGITAIELATGAAPYHKYPPMKVLM** **LTLQNDPPSLETGVQDKEMLKKYGKSFRKMISLCLQKDPEKRPTAAEL** **LRHKFFQKAKNKEFLQEKTLQRAPTISERAKKVRRVPGSSGRLHKTED** **GGWEWSDDEFDEESEEGKAAISQLRSPRVKESISNSELFPTTDPVGTL** **LQVPEQISAHLPQPAGQIATQPTQVSLPPTAEPAKTAQALSSGSGSQE** **TKIPISLVLRLRNSKKELNDIRFEFTPGRDTAEGVSQELISAGLVDGR** **DLVIVAANLQKIVEEPQSNRSVTFKLASGVEGSDIPDDGKLIGFAQLS** **IS**
Native sequence	Amino acids M1 – S527 (end) of human OSR1. Residue M244 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220 and the HA tag (*YPYDVPDYA*). The enzyme has a D164A mutation, which produces a kinase dead enzyme. Residue D164A is equivalent to A407 of the fusion protein.
Protease cleavage	PreScission site (LEVLFQGP) residues 221 – 228

Cloning sites

*Bam*H1 and *Not*1 sites of pGex6P1

Nucleotide sequence of insert

ggatccgccaccATGTACCCATACGATGTGCCAGATTACGCCATGTCC
GAAGACTCGAGCGCCCTGCCCTGGTCCATCAACAGGGACGATTACGAG
CTGCAGGAGGTGATCGGGAGTGGAGCAACTGCTGTAGTCCAAGCAGCT
TATTGTGCCCCTAAAAAGGAGAAAGTGGCAATCAAACGGATAAACCTT
GAGAAATGTCAAACTAGCATGGATGAACTCCTGAAAGAAATTCAAGCC
ATGAGTCAATGCCATCATCCTAATATTGTATCTTACTACACATCTTTT
GTGGTAAAAGATGAGCTGTGGCTTGTCATGAAGCTGCTAAGTGGAGGT
TCTGTTCTGGATATTATTAAGCACATTGTGGCAAAAGGGGAACACAAA
AGTGGAGTCCTAGATGAATCTACCATTGCTACGATACTCCGAGAAGTA
CTGGAAGGGCTGGAATATCTGCATAAAAATGGACAGATCCACAGAGAT
GTGAAAGCTGGAAACATTCTTCTTGGAGAAGATGGCTCAGTACAGATT
GCAGACTTTGGGGTTAGTGCTTTTTTAGCAACTGGTGGTGATATTACC
CGAAATAAAGTGAGAAAGACCTTTGTTGGCACCCCTTGTTGGATGGCA
CCTGAAGTTATGGAACAGGTCCGTGGTTATGATTTCAAAGCTGATATT
TGGAGTTTTGGAATTACAGCAATTGAATTGGCTACAGGGGCGGCTCCT
TATCATAAATATCCACCAATGAAGGTTTTAATGCTGACACTGCAGAAC
GATCCTCCTTCTTTGGAAACTGGTGTTCAAGATAAAGAAATGCTGAAA
AAATATGGAAAATCATTTAGAAAAATGATTTCATTGTGCCTTCAAAAA
GATCCAGAAAAAAGACCAACAGCAGCAGAACTATTAAGGCACAAATTT
TTCCAGAAAGCAAAGAATAAAGAATTTCTTCAAGAAAAAACATTGCAG
AGAGCACCAACCATTTCTGAAAGAGCAAAAAAGGTTCGGAGGGTACCA
GGTTCCAGTGGGCGTCTTCATAAGACAGAGGATGGAGGCTGGGAGTGG
AGTGATGATGAATTTGATGAAGAAAGTGAGGAAGGGAAAGCAGCAATT
TCACAACTCAGGTCTCCCCGAGTGAAAGAATCAATATCAAATTCTGAG
CTCTTTCCAACAACTGATCCTGTGGGTACTTTGCTCCAAGTTCCAGAA
CAGATCTCTGCTCATCTACCTCAGCCAGCTGGGCAGATTGCTACACAG
CCAACTCAAGTCTCTCTCCCACCCACCGCAGAGCCAGCAAAAACAGCT
CAGGCTTTGTCTTCAGGATCAGGTTCACAAGAAACCAAGATCCCAATC
AGTCTAGTACTAAGATTAAGGAATTCCAAAAAAGAACTAAATGATATT
CGATTTGAATTTACTCCTGGGAGAGATACAGCAGAGGGTGTCTCTCAG
GAACTCATTTCTGCTGGCCTGGTCGACGGAAGGGATTTAGTAATAGTG
GCAGCTAATTTGCAGAAAATTGTGGAAGAACCTCAGTCAAATCGATCT
GTCACTTTCAAACTGGCGTCTGGTGTCGAAGGCTCAGATATTCCTGAT
GATGGTAAACTGATAGGATTTGCCCAGCTCAGCATCAGCtaagcggcc
gc