

Certificate of Analysis

OSR1, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 16-048, 16-048-K, 16-048M

Parent Lot # D18HP002N

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: N-terminal GST-tagged, recombinant, human OSR1 full length and N-terminal GST tagged, recombinant, human MO25 α full length, co-expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose. Complex activated *in vivo* with recombinant WNK3. Purity 97% (combined) by SDS-PAGE and Coomassie blue staining. OSR1 MW = 86kDa MO25 α MW = 67kDa.

Specific Activity (Parent lot# D18HP002N): 177U/mg, where one unit of OSR1 activity is defined as 1nmol phosphate incorporated into 125 μ M catchtide per minute at 30°C with a final ATP concentration of 100 μ M.

Formulation: 0.64mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

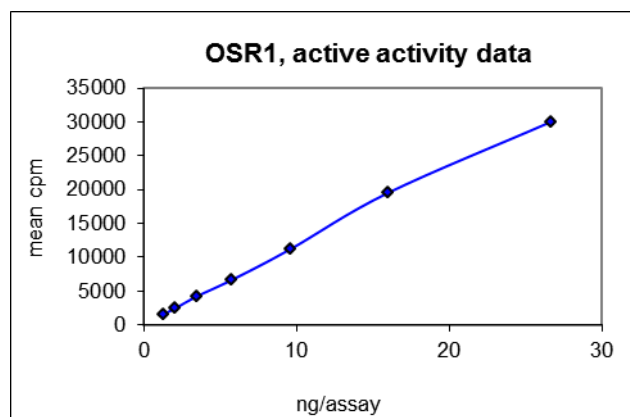
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

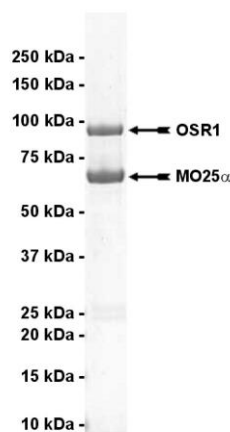
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 1.24–26.67ng of this lot of enzyme phosphorylated 125 μ M catchtide in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed identity as OSR1 and MO25 α with the translated sequences listed on pages three and five.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3 μ g of OSR1, active.

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Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
- 2. Catchtide:** Use at a final assay concentration of 125 μ M. Prepare a 2.5mM stock and add 1.25 μ l of stock per assay point.
- 3. OSR1, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1.24–26.67ng per assay point.
- 4. [γ -³³P]ATP:** 2.5 x MgAc/[γ -³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required).

Assay Procedure (96 well plate format):

1. Add 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 1.25 μ l of catchtide.
3. Add **2.5 μ l (1.24-26.67ng) OSR1, active.**
4. Add 6.25 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture.
6. Incubate for 30 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ l aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1 μ l of 30% phosphoric acid.

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OSR1, active Sequence Information

Protein	Human OSR1
Tags	N-terminal GST
Native sequence	M243 of the recombinant protein is equivalent to M1 of human OSR1.
Accession number	GenBank BC008726.2

Recombinant OSR1 amino acid sequence:

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1  MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61  GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAIQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LVPRGSKEFK GLRRRAQLVR
241 PQMSSEDSSAL PWSINRDDYE LQEVIGSGAT AVVQAAYCAP KKEKVAIKRI NLEKCQTSMD
301 ELLKEIQAMS QCHHPNIVSY YTSFVVKDEL WLVMKLLSGG SVLDIIKHIV AKGEHKSGLV
361 DESTIATILR EVLEGLEYLH KNGQIHRDVK AGNILLGEDG SVQIADFGVS AFLATGGDIT
421 RNKVRKTFVG TPCWMAPEVM EQVRGYDFKA DIWSFGITAI ELATGAAPYH KYPPMKVLM
481 TLQNDPPSLE TGVQDKEMLK KYGKSFRKMI SLCLQKDPEK RPTAAELLRH KFFQKAKNKE
541 FLQEKTLQRA PTISERAKKV RRVPGSSGRL HKTEDGGWEW SDDEFDEESE EGKAAISQLR
601 SPRVKESISN SELFPTTDPV GTLLQVPEQI SAHLPQPAGQ IATQPTQVSL PPTAEPAKTA
661 QALSSGSGSQ ETKIPISLVL RLRNSKKELN DIRFEFTPGR DTAEGVSQEL ISAGLVDGRD
721 LVIVAANLQK IVEEPQSNRS VTFKLASGVE GSDIPDDGKL IGFAQLSIS

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Recombinant OSR1 nucleotide sequence:

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1  atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaaccacac tcgacttctt
61  ttggaatatc ttgaagaaaa atatgaagag catttgatat agcgcgatga aggtgataaa
121 tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatattgat
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361 gattttctta gcaagctacc tgaaatgctg aaaatgttcg aagatcgttt atgtcataaa
421 acatatatta atggtgatca tgtaacccat cctgacttca tgttgatatga cgctcttgat
481 gttgttttat acatggaccc aatgtgcctg gatgcgttcc caaaattagt ttgttttaaa
541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
601 tggcctttgc agggctggca agccacgttt ggtggtggcg accatcctcc aaaatcggat
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1801 tctccccgag tgaaagaatc aatatcaaat tctgagctct ttccaacaac tgatcctgtg
1861 ggtactttgc tccaagttcc agaacagatc tctgctcatc tacctcagcc agctgggcag
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2161 ttagtaatag tggcagctaa tttgcagaaa attgtggaag aacctcagtc aaatcgatct
2221 gtcactttca aactggcatc tgggtgtcgaa ggctcagata ttcttgatga tggtaaactg
2281 ataggatttg ccagctcag catcagctaa

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MO25α Sequence Information

Protein	Human MO25α
Tags	N-terminal GST
Native sequence	M232 of the recombinant protein is equivalent to M1 of human MO25α
Accession number	GenBank NM_016289.2

Recombinant MO25α amino acid sequence:

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1  MSPILGYWKI  KGLVQPTRL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQSMA  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121  DFLSKLPEML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181  KRIEAIPOID  KYLKSSKYIA  WPLQGWQATF  GGGDHPPKSD  LEVLFQGPLG  SMPFFPGKSH
241  KSPADIVKNL  KESMAVLEKQ  DISDKKAeka  TEEVSKNLVA  MKEILYGTNE  KEPQTEAVAQ
301  LAQELYNSGL  LSTLVADLQL  IDFEGKKDVA  QIFNNILRRQ  IGTTRPTVEY  ICTQQNILFM
361  LLKGYESPEI  ALNCGIMLRE  CIRHEPLAKI  ILWSEQFYDF  FRYVEMSTFD  IASDAFATFK
421  DLLTRHKLLS  AEFLEQHYDR  FFSEYEKLLH  SENYVTKRQS  LKLLGELLLD  RHNFTIMTKY
481  ISKPENLKL  MNLLRDKSRN  IQFEAFHVKF  VFVANPNKTQ  PILDILLKNQ  AKLIEFLSKF
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Recombinant MO25α nucleotide sequence:

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1681  atcagggatt  tgaagagacc  agctcagcaa  gaagcttaa
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