

Certificate of Analysis

Ron, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-581, 14-581-K, 14-581M

Parent Lot # D16SP002N

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 Ron amino acids 983–end, expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose followed by size exclusion chromatography. The recombinant product is activated *in vitro* with ATP. Purity 87% by SDS-PAGE and Coomassie blue staining. MW = 74.2kDa.

Formulation: 0.53mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 0.2mM PMSF, 1mM benzamidine, 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.

Specific Activity (Parent lot# D16SP002N): 56U/mg, where one unit of Ron, active activity is defined as 1nmol phosphate incorporated into 250µM GGMEIYFEFMGGKKK per minute at 30°C with a final ATP concentration of 100µM.

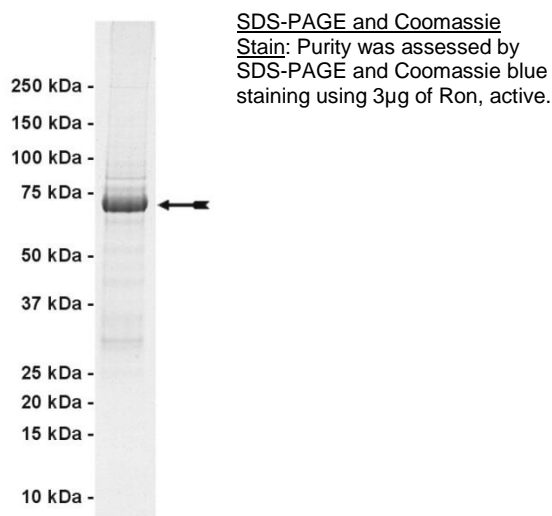
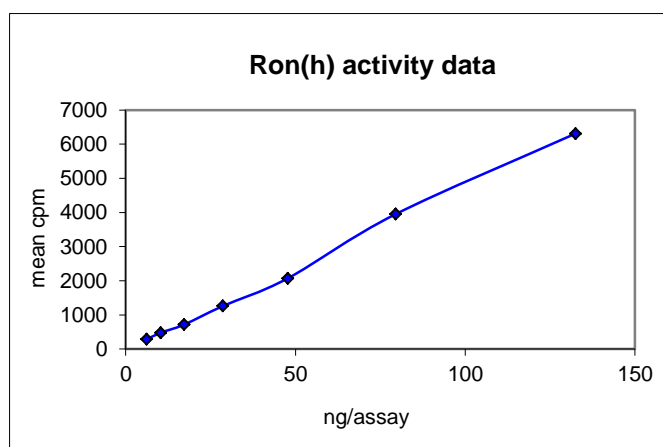
Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

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

Quality Control Testing

Kinase Assay: 6.18–132.50ng of this lot of enzyme phosphorylated 250µM GGMEIYFEFMGGKKK 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 product identity as Ron with the translated sequence listed on page three.



Certificate of Analysis

Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **GGMEDIYFEFMGGKKK:** Use at a final assay concentration of 250 μ M. Prepare a 2.5mM stock and add 2.5 μ l of stock per assay point.
3. **Ron, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 6.18–132.50ng per assay point.
4. **[γ -³³P]ATP:** 2.5 x magnesium acetate/[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 2.5 μ l of GGMEDIYFEFMGGKKK to wells.
3. Add **2.5 μ l (6.18–132.50ng) Ron, active.**
4. Add 5 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 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.

Certificate of Analysis

Ron Sequence Information

<u>Protein</u>	Human Ron
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	R231 of recombinant sequence is equivalent to R983 of native human Ron
<u>Accession number</u>	GenBank NM_002447

Recombinant Ron amino acid sequence:

```

1 MSPILGYWKI KGLVQPTRL L LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61 GDVKLTQ SMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAI PQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LEVL FQGPEF RRRQLVLPN
241 LNDLASLDQT AGATPLPILY SGSDYRSGLA LPAIDGLDST TCVHGASFSD SEDESCVPLL
301 RKESIQLRDL DSALLAEVKD VLIPHERVVT HSDRVIGKGH FGVVYHGEYI DQAQNRIQCA
361 IKSLSRITEM QQVEAFLREG LLMRGLNHPN VLALIGIMLP PEGLPHVLLP YMCHGDLLQF
421 IRSPQRNPTV KDLISFGLQV ARGMEYLAEQ KFVHRDLAAR NCMLDESFTV KVADFGLARD
481 ILDREYYSVQ QHRHARLPVK WMALES LQTY RFTTKSDVWS FGVLLWELLT RGAPPYRHID
541 PFDLTHFLAQ GRRLPQPEYC PDSLYQVMQQ CWEADPAVRP TFRVLVGEVE QIVSALLGDH
601 YVQLPATYMN LGPSTHEMN VRPEQPQFSP MPGNVRRPRP LSEPPRPT
    
```

Recombinant Ron nucleotide sequence:

```

1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaaccac tcgacttctt
61 ttggaatadc ttgaagaaaa atatgaagag catttgtatg agcgcgatga aggtgataaa
121 tggcgaagaa aaaagtttga attgggtttg gagtttccca atcttcctta ttatattgat
181 ggtgatgatta aattaacaca gtctatggcc atcatacgtt atatagctga caagcacaac
241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
301 gatattagat acggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
361 gattttctta gcaagctacc tgaatgctg aaaatgttcg aagatcgttt atgtcataaa
421 acatatttaa atggtgatca tgtaaccat cctgacttca tgttgtatga cgctcttgat
481 gttgttttat acatggacc aatgtgcctg gatgcgttcc caaaattagt ttgtttaaa
541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
601 tggccttttg agggctggca agccacgttt ggtgggtggcg accatcctcc aaaatcggat
661 ctggaagttc tgttcagggg gcccaattc cggaggaagc agctagtctt tctctccaac
721 ctgaatgacc tggcatccct ggaccagact gctggagcca caccctgcc tattctgtac
781 tcgggctctg actacagaag tggccttgca ctccctgcca ttgatggtct ggattccacc
841 acttgtgtcc atggagcatc cttctccgat agtgaagatg aatcctgtgt gccactgctg
901 cggaaagagt ccatccagct aagggacctg gactctgcgc tcttggctga ggtcaaggat
961 gtgctgattc cccatgagcg ggtggtcacc cacagtgacc gagtcattgg caaaggccac
1021 tttggagttg tctaccacgg agaatacata gaccaggccc agaatcgaat ccaatgtgcc
1081 atcaagtcat taagtcgcat cacagagatg cagcagggtgg aggccttctc gcgagagggg
1141 ctgctcatgc gtggcctgaa ccaccgaaat gtgctggctc tcattggtat catgttgcca
1201 cctgagggcc tgccccatgt gctgctgcc tatatgtgcc acggtgacct gctccagttc
1261 atccgctcac ctacagcgaa ccccaccgtg aaggacctca tcagctttgg cctgcaggta
1321 gcccgggca tggagtacct ggacagagcag aagtttgtgc acagggacct ggctgcgagg
1381 aactgcatgc tggacgagtc attcacagtc aaggtggctg actttggttt ggcccgagc
1441 atcctggaca gggagtacta tagtgttcaa cagcatcgcc acgctcgctt acctgtgaag
1501 tggatggcgc tggagagcct gcagacctat agatttacca ccaagtctga tgtgtggtca
1561 tttggtgtgc tgctgtggga actgctgaca cggggtgcc caccataccg ccacattgac
1621 ccttttgacc ttaccactt cctggcccag ggtcggcgcc tgccccagcc tgagtattgc
1681 cctgattctc tgtaccaagt gatgcagcaa tgctgggagg cagaccagc agtgcgacc
    
```

Certificate of Analysis

```
1741 accttcagag tactagtggg ggaggtggag cagatagtgt ctgcactgct tggggaccat
1801 tatgtgcagc tgccagcaac ctacatgaac ttgggccccca gcacctcgca tgagatgaat
1861 gtgctccag aacagccgca gttctcacc atgccagga atgtacgccg gccccggcca
1921 ctctcagagc ctctcggcc cacttga
```

Reviewed and approved by site quality representative.

Unless otherwise stated in our catalogue or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

© 2014 **Eurofins Pharma Discovery Services UK Limited** is an independent member of Eurofins Discovery Services.