

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.

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

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.

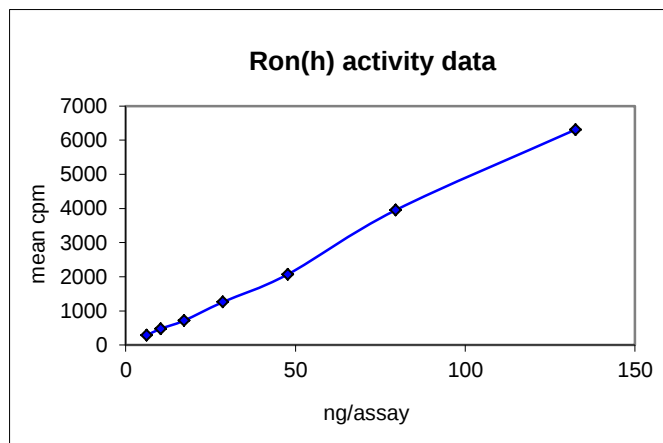
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

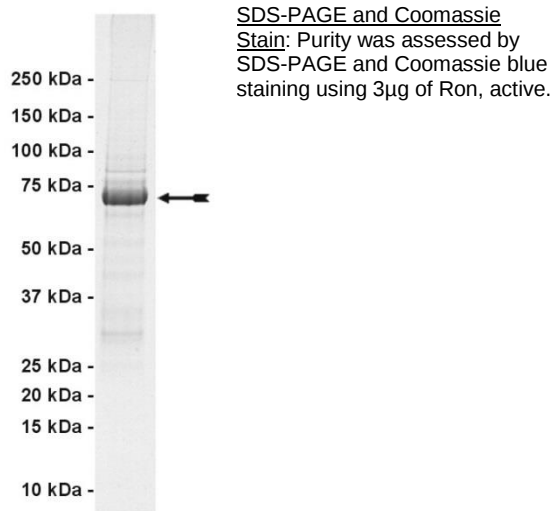
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 6.18–132.50ng of this lot of enzyme phosphorylated 250µM GGMEDIYFEFMGGKKK 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.



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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.

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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:

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1  MSPILGYWKI KGLVQPTRL L LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61  GDVKLTQSM A IIRYIADKH N MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAI PQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LEVLFQGPEF RRKQLVLPPN
241 LNDLASLDQT AGATPLPILY SGSDYRSGLA LPAIDGLDST TCVHGASFSD SEDESCVPLL
301 RKESIQLRDL DSALLAEVKD VLIPHERVVT HSDRVIGKGH FGVVYHGEYI DQAQNRIQCA
361 IKSLSRITEM QQVEAFLREG LLMRGLNHPN VLALIGIMLP PEGLPHVLLP YMCHGDL LQF
421 IRSPQRNPTV KDLISFGLQV ARGMEYLA EQ KFVHRDLAAR NCMLDESFTV KVADFGLARD
481 ILDREYYSVQ QHRHARLPVK WMALES LQTY RFTTKSDVWS FGVLLWELLT RGAPPYRHID
541 PFDLTHFLAQ GRRLPQPEYC PDSLYQVMQ Q CWEADPAVRP TFRVLVGEVE QIVSALLGDH
601 YVQLPATYMN LGPSTHEMN VRPEQPQFSP MPGNVRRPRP LSEPPRPT

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Recombinant Ron nucleotide sequence:

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1  atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaaccacac tcgacttctt
61  ttggaatata ttgaagaaaa atatgaagag catttgatat agcgcgatga aggtgataaa
121  tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatatgtat
181  ggtgatgtta aattaacaca gtctatggcc atcatagctt atatagctga caagcacaac
241  atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
301  gatattagat acggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
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1621 ctttttgacc ttaccactt cctggcccag ggtcggcgcc tgcccagacc tgagtattgc
1681 cctgattctc tgtaccaagt gatgcagcaa tgctgggagg cagaccagc agtgcgaccc

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1861 gtgcgtccag aacagccgca gttctcacc atgccaggga atgtacgccg gccccggcca
1921 ctctcagagc ctctcggcc cacttga
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