Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ RON

macrophage stimulating 1 receptor

Recombinant Human Active Protein Kinase

HGNC Symbol: MST1R

Synonyms: PTK8, CDw136, SEA, CD136

Product No.: 0889-0000-1

Lot: 009

Description: Human RON, internal fragment, amino acids L_{1052}-E_{1370} (as in NCBI/Protein entry NP_002438.2), activated, N-terminal GST-HIS_6 fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: RON Lot 009, was confirmed as RON by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 64,998 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: in vitro auto activation

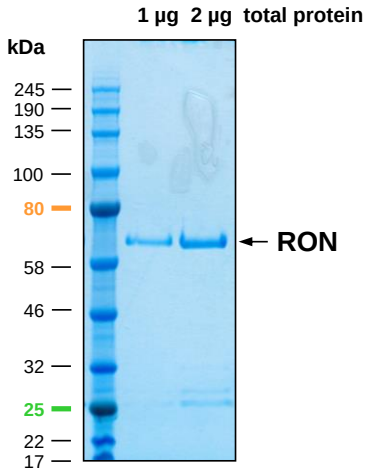
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

Storage temperature: -80°C
For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10µl are not advisable. Avoid repeated freeze-thaw cycles!

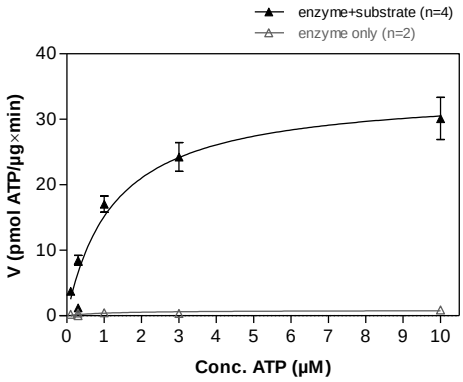
Protein concentration: 0.374 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:
Specific kinase activity (P_i transfer): 34 pmol/µg × min
ATP-K_M: 1.3 µM

RON Lot 009:
Coomassie stain



RON Lot 009:
Determination of V_{max} and K_M value for ATP



- Assay conditions:
  60 mM HEPES-NaOH, pH 7.5
  3 mM $MgCl_2$
  3 mM $MnCl_2$
  3 µM Na-orthovanadate
  1.2 mM DTT
  50 µg/ml $PEG_{20.000}$
  ATP (variable)
  Substrate: TRK-C derived peptide 5 µg/ml
  Kinase: 1.0 µg/ml
- Filter binding assay

MSIP membrane (Millipore)

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GST-RON Recombinant Fusion Protein Amino Acid Sequence

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   1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID  60
  61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV 120
 121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK 180
 181 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD PMGHHHHHHG RDSLEVLFQG 240
 241 PLAMLRKESI QLRDLDSALL AEVKDVLIPH ERVVTHSDRV IGKGHFGVVY HGEYIDQAQN 300
 301 RIQCAIKSLS RITEMQQVEA FLREGLLMRG LNHPNVLALI GIMLPPEGLP HVLLPYMCHG 360
 361 DLLQFIRSPQ RNPTVKDLIS FGLQVARGME YLAEQKFVHR DLAARNCMLD ESFTVKVADF 420
 421 GLARDILDRE YYSVQQHRHA RLPVKWMALE SLQTYRFTTK SDVWSFGVLL WELLTRGAPP 480
 481 YRHIDPFDLT HFLAQGRRLP QPEYCPDSLY QVMQQCWEAD PAVRPTFRVL VGEVEQIVSA 540
 541 LLGDHYVQLP ATYMNLGPST SHE                                         600
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1-218: GST **Red**: HIS6-tag **Green**: 3C cleavage site **blue**: RON fragment

RON wt[1] Amino Acid Sequence

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   1 MELLPPLPQS FLLLLLLPAK PAAGEDWQCP RTPYAASRDF DVKYVVPSFS AGGLVQAMVT  60
  61 YEGDRNESAV FVAIRNRLHV LGPDLKSVQS LATGPAGDPG CQTCAACGPG PHGPPGDTDT 120
 121 KVLVLDPALP ALVSCGSSLQ GRCFLHDLEP QGTAVHLAAP ACLFSAHHNR PDDCPDCVAS 180
 181 PLGTRVTVVE QGQASYFYVA SSLDAAVAAS FSPRSVSIRR LKADASGFAP GFVALSVLPK 240
 241 HLVSYSIEYV HSFHTGAFVY FLTVQPASVT DDPSALHTRL ARLSATEPEL GDYRELVLDC 300
 301 RFAPKRRRRG APEGGQPYPV LRVAHSAPVG AQLATELSIA EGQEVLFGVF VTGKDGGPGV 360
 361 GPNSVVCAFP IDLLDTLIDE GVERCCESPV HPGLRRGLDF FQSPSFCPNP PGLEALSPNT 420
 421 SCRHFPLLVS SSFSRVDLFN GLLGPVQVTA LYVTRLDNVT VAHMGTMDGR ILQVELVRSL 480
 481 NYLLYVSNFS LGDSGQPVQR DVSRLGDHLL FASGDQVFQV PIQGPGCRHF LTCGRCLRAW 540
 541 HFMGCGWCGN MCGQQKECPG SWQQDHCPPK LTEFHPHSGP LRGSTRLTLC GSNFYLHPSG 600
 601 LVPEGTHQVT VGQSPCRPLP KDSSKLRPVP RKDFVEEFEC ELEPLGTQAV GPTNVSLTVT 660
 661 NMPPGKHFRV DGTSVLRGFS FMEPVLIAVQ PLFGPRAGGT CLTLEGQSLS VGTSRAVLVN 720
 721 GTECLLARVS EGQLLCATPP GATVASVPLS LQVGGAQVPG SWTFQYREDP VVLSISPNCG 780
 781 YINSHITICG QHLTSAWHLV LSFHDGLRAV ESRCERQLPE QQLCRLPEYV VRDPQGWVAG 840
 841 NLSARGDGAA GFTLPGFRFL PPPHPPSANL VPLKPEEHAI KFEYIGLGAV ADCVGINVTV 900
 901 GGESCQHEFR GDMVVCPLPP SLQLGQDGAP LQVCVDGECH ILGRVVRPGP DGVPQSTLLG 960
 961 ILLPLLLLVA ALATALVFSY WWRRKQLVLP PNLNDLASLD QTAGATPLPI LYSGSDYRSG 1020
1021 LALPAIDGLD STTCVHGASF SDSEDESCVP LLRKESIQLR DLDSALLAEV KDVLIPHERV 1080
1081 VTHSDRVIGK GHFGVVYHGE YIDQAQNRIQ CAIKSLSRIT EMQQVEAFLR EGLLMRGLNH 1140
1141 PNVLALIGIM LPPEGLPHVL LPYMCHGDLL QFIRSPQRNP TVKDLISFGL QVARGMEYLA 1200
1201 EQKFVHRDLA ARNCMLDESF TVKVADFGLA RDILDREYYS VQQHRHARLP VKWMALESLQ 1260
1261 TYRFTTKSDV WSFGVLLWEL LTRGAPPYRH IDPFDLTHFL AQGRRLPQPE YCPDSLYQVM 1320
1321 QQCWEADPAV RPTFRVLVGE VEQIVSALLG DHYVQLPATY MNLGPSTSHE MNVRPEQPQF 1380
1381 SPMPGNVRRP RPLSEPPRPT                                            1440
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blue: RON sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_002438.2

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