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RON, Active

Recombinant protein expressed in Sf9 cells

Catalog # M58-11G

Lot # L076-2

Product Description

Recombinant human RON (983-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_002447.

Gene Aliases

MST1R, PTK8, CDw136

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.25mM DTT, 0.1mM EGTA, 0.1mM EDTA, 0.1mM PMSF, 25% glycerol.

Storage and Stability

Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

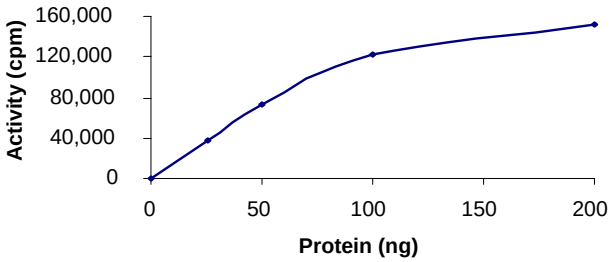
Scientific Background

RON is a macrophage stimulating 1 receptor c-met-related tyrosine kinase (1). RON receptor tyrosine kinase interacts with HYAL2 receptor protein, rendering it functionally inactive. HYAL2 is a candidate tumor-suppressor glycosylphosphatidylinositol-anchored cell-surface protein that serves as an entry receptor for jaagsiekte sheep retrovirus, a virus that causes contagious lung cancer in sheep that is morphologically similar to human bronchioloalveolar carcinoma. It was shown that RON liberated from the association with HYAL2 becomes functionally active and activates the Akt and mitogen-activated protein kinase pathways (2).

References

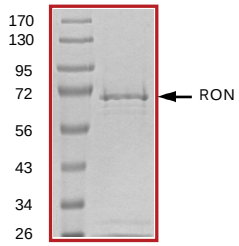
1. Wang, M.H. et al: Identification of the ron gene product as the receptor for the human macrophage stimulating protein. Science, 1994; 266 (5182): 117-9.
2. Li, B,Q, et al: "Macrophage-stimulating protein activates Ras by both activation and translocation of SOS nucleotide exchange factor. Biochem. Biophys. Res. Commun.1995; 216 (1): 110-8.

Specific Activity



The specific activity of RON was determined to be **81 nmol /min/mg** as per activity assay protocol.

Purity



The purity of RON was determined to be **>85%** by densitometry, approx. MW **71kDa**.

RON, Active

Recombinant protein expressed in Sf9 cells

Catalog Number	M58-11G
Specific Activity	81 nmol/min/mg
Specific Lot Number	L076-2
Purity	>85%
Concentration	0.1 µg/µl
Stability	1yr At –70°C from date of shipment
Storage & Shipping	Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: M58-11G)

Active RON (0.1μg/μl) diluted with Kinase Dilution Buffer VII (Catalog #: K27-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active RON for optimal results).

Kinase Dilution Buffer VII (Catalog #: K27-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with final 5% glycerol and 50ng/μl BSA solution.

Kinase Assay Buffer I (Catalog #: K01-09)

Buffer components: 25mM MOPS, pH 7. 2, 12.5mM β-glycerol-phosphate, 25mM $MgC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[^{33}P]-ATP Assay Cocktail

Prepare 250μM [^{33}P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150μl of 10mM ATP Stock Solution (Catalog #: A50-09), 100μl [^{33}P]-ATP (1mCi/100μl), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 1ml aliquots at –20°C.

10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 200μl aliquots at –20°C.

Substrate (Catalog #: A16-58)

Axltide synthetic peptide substrate (KKSRGDYMTMQIG) diluted in distilled H_2O to a final concentration of 1mg/ml.

Assay Protocol

Step 1. Thaw [^{33}P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.

Step 2. Thaw the Active RON, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.

Step 3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20μl:

- **Component 1.** 10μl of diluted Active RON (Catalog #M58-11G)
- **Component 2.** 5μl of 1mg/ml stock solution of substrate (Catalog #A16-58)
- **Component 3.** 5μl distilled H_2O (4°C)

Step 4. Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H_2O.

Step 5. Initiate the reaction by the addition of 5 μl [^{33}P]-ATP Assay Cocktail bringing the final volume up to 25μl and incubate the mixture in a water bath at 30°C for 15 minutes.

Step 6. After the 15 minute incubation period, terminate the reaction by spotting 20 μl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.

Step 7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H_2O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.

Step 8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.

Step 9. Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [P^{33}]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 μl [^{33}P]-ATP / pmoles of ATP (in 5 μl of a 250 μM ATP stock solution, i.e., 1250 pmoles)

Kinase Specific Activity (SA) (pmol/min/μg or nmol/min/mg)

Corrected cpm from reaction / [(SA of ^{33}P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)]*[(Reaction Volume) / (Spot Volume)]

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