

KSR1 (A635F), Active

Recombinant human protein expressed in Sf9 cells

Catalog # K07-12G

Lot # Z1156-4

Product Description

Recombinant human KSR1 (A635F) (431-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The protein accession number is [Q8I1V5](#).

Gene Aliases

KSR; RSU2

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 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.

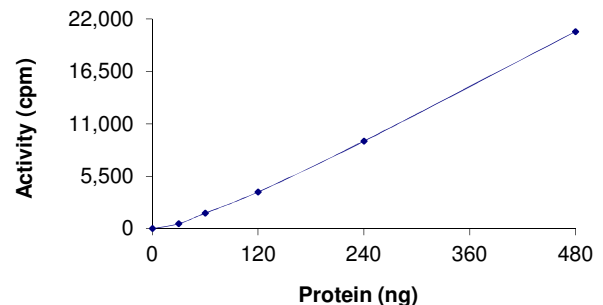
Scientific Background

KSR1 or Kinase Suppressor of Ras 1 interacts with various kinases of the Raf/MEK/extracellular signal-regulated kinase pathway to enhance its activation. KSR1 is regulated in response to a specific mode of dimerization of its kinase domain, which is termed the side-to-side dimer, whereas KSR1 also participates in forming side-to-side heterodimers with RAF and this can trigger RAF activation. KSR1 has an essential protective role in the intestinal epithelial cell during inflammation through activation of cell survival pathways (1). KSR1 functions as a scaffold that enhances iNOS activity and is crucial for the pulmonary response to *P. aeruginosa* infection (2).

References

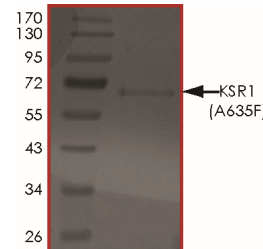
1. Yan, et.al: Kinase suppressor of Ras-1 protects intestinal epithelium from cytokine-mediated apoptosis during inflammation. *J. Clin. Invest.* 114: 1272-1280, 2004.
2. Zhang, et.al: Kinase suppressor of Ras-1 protects against pulmonary *Pseudomonas aeruginosa* infections. *Nature Med.* 17: 341-346, 2011.

Specific Activity



The specific activity of KSR1 (A635F) was determined to be **2.8 nmol/min/mg** as per activity assay protocol.

Purity



The purity of KSR1 (A635F) was determined to be **>80%** by densitometry, approx. MW **66 kDa**.

KSR1 (A635F), Active

Recombinant human protein expressed in Sf9 cells

Catalog #	K07-12G
Specific Activity	2.8 nmol/min/mg
Lot #	Z1156-4
Purity	>80%
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.

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: K07-12G)

Active KSR1 (A635F) (0.1µg/µl) diluted with Kinase Dilution Buffer III (Catalog #: K23-09) 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 KSR1 (A635F) for optimal results).

Kinase Dilution Buffer IV (Catalog #: K23-09)

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

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

Buffer components: 25mM MOPS, pH 7. 2, 12.5mM β-glycerol-phosphate, 25mM MgCl₂, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[³³P]-ATP Assay Cocktail

Prepare 250µM [³³P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [³³P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer II (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 II (Catalog #: K02-09). Store 200µl aliquots at -20°C.

Substrate (Catalog #: C50-58)

CREBtide synthetic peptide substrate (KRREILSRPSYR) diluted in distilled H₂O to a final concentration of 1mg/ml.

Assay Protocol

- Step 1.** Thaw [³³P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active KSR1 (A635F), 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 KSR1 (A635F) (Catalog #K07-12G)
 - Component 2.** 5µl of 1mg/ml stock solution of substrate (Catalog #C50-58)
 - Component 3.** 5µl distilled H₂O (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 H₂O.
- Step 5.** Initiate the reaction by the addition of 5 µl [³³P]-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 H₂O) 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 [³³P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 µl [³³P]-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 ³³P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]

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