SIK3, Active
Human recombinant protein expressed in Sf9 cells

Catalog # S12-11G
Lot # L2160-10

Product Description
Recombinant human SIK3 (1-307) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is BC128510.

Gene Aliases
QSK; SIK-3; L19; FLJ12240; KIAA0999

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
SIK3 (QSK) is a serine/threonine-protein kinase, belongs to QIK subfamily. The phosphorylation of SIK3 by LKB1 through the 14-3-3 binding enhances its catalytic activity and leads its localization to punctate structures within the cytoplasm (1). Overexpression of SIK3 promotes G1/S cell cycle progression with ovarian cancer (2). There are two sites (H331L and A1103V) were mutated at significant frequency in breast cancer (3). SIK3 is a novel tumor-associated antigen (TAA).

References

Specific Activity
The specific activity of SK3 was determined to be 120 nmol/min/mg as per activity assay protocol.

Purity
The purity of SIK3 was determined to be >90% by densitometry, approx. MW 62kDa.

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Catalog # S12-11G
Specific Activity 120 nmol/min/mg
Lot # L2160-10
Purity >90%
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 #: S12-11G)

Active SIK3 (0.1µg/µl) was 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 SIK3 for optimal results).

**Kinase Dilution Buffer III** (Catalog #: K23-09)

Kinase Assay Buffer I (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 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 distilled H₂O to a final concentration of 1mg/ml.

**Substrate** (Catalog #: A11-58)

AMARA Peptide substrate (AMARAASAAALARRR) 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 SIK3, 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 SIK3 (Catalog #S12-11G)
- **Component 2.** 5µl of 1mg/ml stock solution of substrate (Catalog #A11-58)
- **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 [P³³]-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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