

STK19, Active

Recombinant full-length protein expressed in Sf9 cells

Catalog # S35-10G

Lot # F734-2

Product Description

Recombinant full-length human STK19 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The STK19 gene accession number is [NM_004197](#).

Gene Aliases

D6S60; D6S60E; G11; HLA-RP1; RP1

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

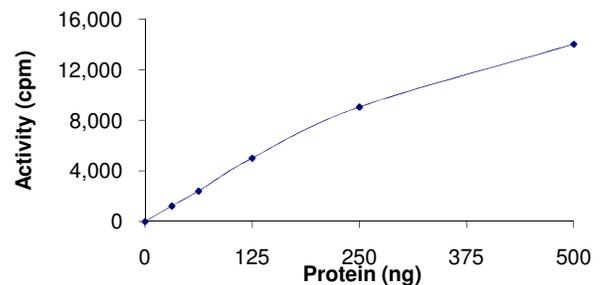
STK19 is a member of the serine/threonine kinase family that shows a manganese-dependent protein kinase activity and phosphorylates α -casein at ser/thr residues and histone at ser residues. STK19 contains a positively charged putative nuclear localization signal, multiple potential phosphorylation sites, 2 amidation sites, 9 potential N-myristoylation sites, and no N-glycosylation sites. STK19 has been shown to localize predominantly in the nucleus (1). The phosphorylation of STK19 is thought to be involved in transcriptional regulation. STK19 localizes to the major histocompatibility complex (MHC) class III region on chromosome 6 (2).

References

- Gomez-Escobar, N. et.al: The G11 gene located in the major histocompatibility complex encodes a novel nuclear serine/threonine protein kinase. J. Biol. Chem. 273: 30954-30960, 1998.
- Shen, L. et.al: Structure and genetics of the partially duplicated gene RP located immediately upstream of the complement C4A and the C4B genes in the HLA class III region: molecular cloning, exon-intron structure, composite retroposon, and breakpoint gene duplication. J. Biol. Chem. 269: 8466-8476, 1994.

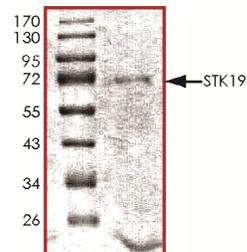
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Specific Activity



The specific activity of STK19 was determined to be **2 nmol/min/mg** as per activity assay protocol.

Purity



The purity of STK19 was determined to be **>70%** by densitometry, approx. MW **70kDa**.

STK19, Active

Recombinant full-length human protein expressed in Sf9 cells

Catalog Number	S35-10G
Specific Activity	2 nmol/min/mg
Specific Lot Number	F734-2
Purity	>70%
Concentration	0.05 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 #: S35-10G)

Active STK19 (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 STK19 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 Kinase Assay Buffer I (Catalog #: K01-09). 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 STK19, 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 STK19 (Catalog # S35-10G)
 - 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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