

## LOK, Active

Recombinant human protein expressed in Sf9 cells

**Catalog # S29-11G**

Lot # 1099-2

### Product Description

Recombinant human LOK (1-348) was expressed in Sf9 cells using a N-terminal GST tag. The gene accession number is [NM\\_005990](#).

### Gene Aliases

STK10, PRO2729

### 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  $-70^{\circ}\text{C}$ . 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.

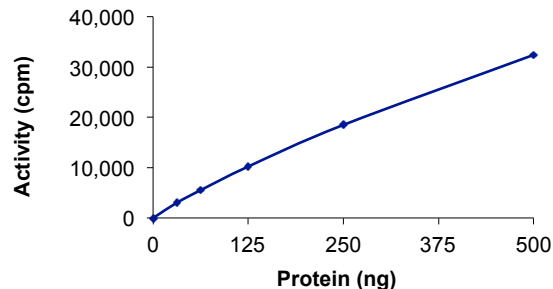
### Scientific Background

LOK is a member of the Ste20 family of serine/threonine protein kinases, and is similar to several known polo-like kinase kinases. LOK can associate with and phosphorylate polo-like kinase 1, and overexpression of a kinase-dead version of the protein interferes with normal cell cycle progression (1). LOK can also negatively regulate interleukin 2 expression in T-cells via the mitogen activated protein kinase kinase 1 pathway. MEKK1 and LOK have opposing roles in regulating the enhancer element of the IL-2 gene during T-cell activation. While MEKK1 is responsible for the CD28 signalling pathway that activates the CD28 response element, co-expression of LOK attenuates these effects (2).

### References

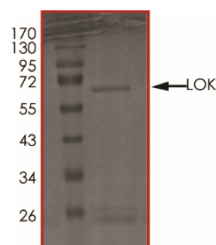
1. Walter, S A. et al: Stk10, a new member of the polo-like kinase kinase family highly expressed in hematopoietic tissue. *J Biol Chem*. 2003 May 16;278(20):18221-8.
2. Tao, L. et al: Opposing roles of serine/threonine kinases MEKK1 and LOK in regulating the CD28 responsive element in T-cells. *Biochem J*. 2002 Apr 1;363(Pt 1):175-82.

### Specific Activity



The specific activity of LOK was determined to be **2.8 nmol /min/mg** as per activity assay protocol.

### Purity



The purity of LOK was determined to be **>75%** by densitometry. Approx. MW **~65kDa**.

## LOK, Active

Recombinant protein expressed in Sf9 cells

Catalog #	S29-11G
Specific Activity	2.8 nmol/min/mg
Lot #	1099-2
Purity	>75%
Concentration	0.1 µg/µl
Stability	1yr at $-70^{\circ}\text{C}$ from date of shipment
Storage & Shipping	Store product at $-70^{\circ}\text{C}$ . 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 #: S29-11G)

Active LOK (0.1µg/µl) diluted with Kinase Dilution Buffer (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 LOK 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 MgCl<sub>2</sub>, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

### [<sup>33</sup>P]-ATP Assay Cocktail

Prepare 250µM [<sup>33</sup>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 [<sup>33</sup>P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer (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 (Catalog #: K01-09). Store 200µl aliquots at -20°C.

### Substrate (Catalog #: A16-58)

Axltide synthetic peptide substrate (KKSREGDYMTMQIG) diluted in distilled H<sub>2</sub>O to a final concentration of 1mg/ml.

## Assay Protocol

- Step 1.** Thaw [<sup>33</sup>P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active LOK, Kinase Assay Buffer, Substrate and Enzyme 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 LOK (Catalog # S29-11G)
  - Component 2.** 5µl of 1 mg/ml stock solution of substrate (Catalog # A16-58)
  - Component 3.** 5µl distilled H<sub>2</sub>O
- 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<sub>2</sub>O.
- Step 5.** Initiate the reaction by the addition of 5µl [<sup>33</sup>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 20µl 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<sub>2</sub>O) 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 [<sup>33</sup>P]-ATP Specific Activity (SA) (cpm/pmol)

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

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

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

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