LOK (1-348), active, GST-tagged, human Precisio® Kinase recombinant, expressed in Sf9 cells

Catalog Number L3794
Lot Number 060M0858
Storage Temperature –70 °C

Synonyms: STK10, PRO2729

Product Description
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 over-expression 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 IL2 gene during T cell activation. While MEKK1 is responsible for the CD28 signalling pathway that activates the CD28 response element, coexpression of LOK attenuates these effects.2

This recombinant product was expressed by baculovirus in Sf9 insect cells using an N-terminal GST-tag. The gene accession number is NM 005990. It is supplied in 50 mM Tris-HCl, pH 7.5, with 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, and 25% glycerol.

Molecular mass: ~65 kDa

Purity: ≥70% (SDS-PAGE, see Figure 1)

Specific Activity: 2–4 nmole/min/mg (see Figure 2)

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
The product ships on dry ice and storage at –70 °C is recommended. After opening, aliquot into smaller quantities and store at –70 °C. Avoid repeated handling and multiple freeze/thaw cycles.
Kinase Solution – Dilute the active LOK (0.1 µg/µl) with Kinase Dilution Buffer to the desired concentration. **Note:** The lot-specific specific activity plot may be used as a guideline (see Figure 2). It is recommended that the researcher perform a serial dilution of active LOK kinase for optimal results.

10 mM ATP Stock Solution – Dissolve 55 mg of ATP in 10 ml of Kinase Assay Buffer. Store in 200 µl aliquots at −20 °C.

γ-32P-ATP Assay Cocktail (250 µM) – Combine 5.75 ml of Kinase Assay Buffer, 150 µl of 10 mM ATP Stock Solution, 100 µl of γ-32P-ATP (1 mCi/100 µl). Store in 1 ml aliquots at −20 °C.

Substrate Solution – Dissolve the synthetic peptide substrate (KKSRGDYMTMQIG) in water at a final concentration of 1 mg/ml.

1% phosphoric acid solution – Dilute 10 ml of concentrated phosphoric acid to a final volume of 1 L with water.

**Kinase Assay**

This assay involves the use of the 32P radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Thaw the active LOK, Kinase Assay Buffer, Substrate Solution, and Kinase Dilution Buffer on ice. The γ-32P-ATP Assay Cocktail may be thawed at room temperature.
2. In a pre-cooled microcentrifuge tube, add the following solutions to a volume of 20 µl:
   - 10 µl of Kinase Solution
   - 5 µl of Substrate Solution
   - 5 µl of cold water (4 °C)
3. Set up a blank control as outlined in step 2, substituting 5 µl of cold water (4 °C) for the Substrate Solution.
4. Initiate each reaction with the addition of 5 µl of the γ-32P-ATP Assay Cocktail, bringing the final reaction volume to 25 µl. Incubate the mixture in a water bath at 30 °C for 15 minutes.
5. After the 15 minute incubation, stop the reaction by spotting 20 µl of the reaction mixture onto an individually precut strip of phosphocellulose P81 paper.
6. Air dry the precut P81 strip and sequentially wash in the 1% phosphoric acid solution with constant gentle stirring. It is recommended the strips be washed a total of 3 times of ~10 minutes each.
7. Set up a radioactive control to measure the total γ-32P-ATP counts introduced into the reaction. Spot 5 µl of the γ-32P-ATP Assay Cocktail on a precut P81 strip. Dry the sample for 2 minutes and read the counts. Do not wash this sample.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
9. Determine the corrected cpm by subtracting the blank control value (see step 3) from each sample and calculate the kinase specific activity

**Calculations:**

1. **Specific Radioactivity (SR) of ATP (cpm/nmole)**
   
   \[ SR = \frac{cpm \text{ of } 5 \mu l \text{ of } \gamma-32P-ATP \text{ Assay Cocktail}}{nmole \text{ of ATP}} \]
   
   \[ cpm = value \text{ from control (step 7)} \]
   
   \[ nmole = 1.25 \text{ nmole (5 } \mu l \text{ of 250 } \mu M \text{ ATP Assay Cocktail)} \]

2. **Specific Kinase Activity (SA) (nmole/min/mg)**
   
   \[ \text{nmole/min/mg} = \frac{\Delta \text{cpm} \times (25/20)}{SR \times E \times T} \]

   \[ SR = \text{specific radioactivity of the ATP (cpm/nmole ATP)} \]
   
   \[ \Delta \text{cpm} = \text{cpm of the sample – cpm of the blank (step 3)} \]
   
   \[ 25 = \text{total reaction volume} \]
   
   \[ 20 = \text{spot volume} \]
   
   \[ T = \text{reaction time (minutes)} \]
   
   \[ E = \text{amount of enzyme (mg)} \]

**References**


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