

## Product Information

**STK3, active, GST-tagged, human  
PRECISIO® Kinase  
recombinant, expressed in Sf9 cells**

Catalog Number **S6573**  
Storage Temperature  $-70^{\circ}\text{C}$

Synonyms: KRS1, MST2

### Product Description

STK3, also known as MST2, encodes a protein of 491 amino acids which contains an N-terminal catalytic domain characteristic of STKs.<sup>1</sup> STK3 and STK4 share 94% amino acid identity in the catalytic domain and 78% identity overall. RAF1 has been shown to counteract apoptosis by suppressing the activation of mammalian sterile 20-like kinase (MST2). STK3 is a kinase that is activated by the proapoptotic agents, strausporine and FAS ligand.<sup>2</sup> STK3 activation presumably allows cells to resist unfavorable environmental conditions.

This recombinant product was expressed by baculovirus in Sf9 insect cells using an N-terminal GST-tag. The gene accession number is BC 010640. 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: ~87 kDa

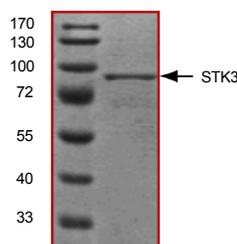
### Precautions and Disclaimer

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.

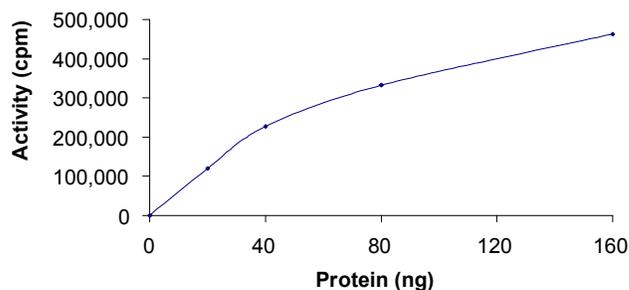
### Storage/Stability

The product ships on dry ice and storage at  $-70^{\circ}\text{C}$  is recommended. After opening, aliquot into smaller quantities and store at  $-70^{\circ}\text{C}$ . Avoid repeated handling and multiple freeze/thaw cycles.

**Figure 1.**  
SDS-PAGE Gel of Typical Lot:  
 $\geq 70\%$  (SDS-PAGE, densitometry)



**Figure 2.**  
Specific Activity of Typical Lot:  
235–319 nmole/min/mg



### Procedure

#### Preparation Instructions

Kinase Assay Buffer – 25 mM MOPS, pH 7.2, 12.5 mM glycerol 2-phosphate, 25 mM  $\text{MgCl}_2$ , 5 mM EGTA, and 2 mM EDTA. Just prior to use, add DTT to a final concentration of 0.25 mM.

Kinase Dilution Buffer – Dilute the Kinase Assay Buffer 5-fold with a 50 ng/ $\mu\text{l}$  BSA solution.

Kinase Solution – Dilute the active STK3 (0.1 µg/µl) with Kinase Dilution Buffer to the desired concentration.

Note: The specific activity plot may be used as a guideline (see Figure 2). It is recommended the researcher perform a serial dilution of active STK3 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-<sup>32</sup>P-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-<sup>32</sup>P-ATP (1 mCi/100 µl). Store in 1 ml aliquots at –20 °C.

Substrate Solution – Dissolve the synthetic peptide substrate myelin basic protein (MBP) 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 <sup>32</sup>P radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Thaw the active STK3, Kinase Assay Buffer, Substrate Solution, and Kinase Dilution Buffer on ice. The γ-<sup>32</sup>P-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
  - 10 µl of Substrate Solution
3. Set up a blank control as outlined in step 2, substituting 10 µl of cold water (4 °C) for the Substrate Solution.
4. Initiate each reaction with the addition of 5 µl of the γ-<sup>32</sup>P-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 pre-cut strip of phosphocellulose P81 paper.

6. Air dry the pre-cut 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-<sup>32</sup>P-ATP counts introduced into the reaction. Spot 5 µl of the γ-<sup>32</sup>P-ATP Assay Cocktail on a pre-cut 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)

$$SR = \frac{\text{cpm of 5 } \mu\text{l of } \gamma\text{-}^{32}\text{P-ATP Assay Cocktail}}{\text{nmole of ATP}}$$

cpm – value from control (step 7)

nmole – 1.25 nmole (5 µl of 250 µM 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 = specific radioactivity of the ATP (cpm/nmole ATP)

Δcpm = cpm of the sample – cpm of the blank (step 3)

25 = total reaction volume

20 = spot volume

T = reaction time (minutes)

E = amount of enzyme (mg)

#### References

1. Creasy, C.L. et al., Cloning and characterization of a member of the MST subfamily of Ste20-like kinases. *Gene*, **167**, 303-306 (1995).
2. Lee, K.K. et al., MST, a physiological caspase substrate, highly sensitizes apoptosis both upstream and downstream of caspase activation. *J. Biol. Chem.*, **276**, 19276-19285 (2001).

PRECISIO is a registered trademark of Sigma-Aldrich Co. LLC.

JB,JR,MAM 04/12-1