

Product Specification

STK3, active

(Full-length recombinant protein expressed in Sf9 cells)

Catalog # 7751-5

Lot # _____

Aliquot size: 5 µg protein in 50 µl

Specific activity: 277 nmol/min/mg

Quality Control Analysis

Activity assessment

STK3 protein (~100 ng/µl concentration) was diluted to 20ng/µl with assay dilution buffer (4 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 4 mM MgCl₂, 0.05 mM DTT and 40ng/µl BSA), followed by 2-fold serial dilutions, and then the 10µl diluted proteins were used to phosphorylate the MBP in the following assay condition:

10 µl diluted STK3 protein

10 µl MBP (2 mg/ml stock)

5 µl [³²P] ATP mixture (250 µM ATP, 0.16 µCi/µl in 4x assay dilution buffer)

The various reaction components, except [³²P] ATP, were incubated at 30°C and the reaction started by the addition of [³²P] ATP. After 15 minutes, the reaction was terminated by spotting 20 µl of the reaction mixture onto a phosphocellulose P81 paper. The P81 paper was dried and washed several times in 1% phosphoric acid prior to counting in the presence of scintillation fluid in a scintillation counter. The actual counts, using various dilutions of the enzyme in the assay, are shown in Fig. 1.

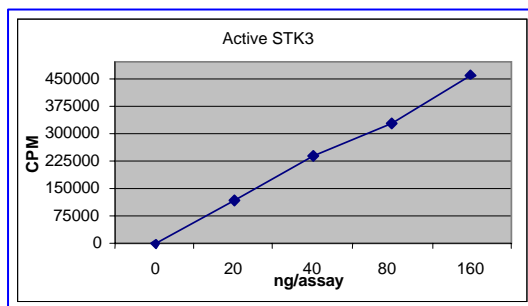


Fig. 1 STK3 activity assay

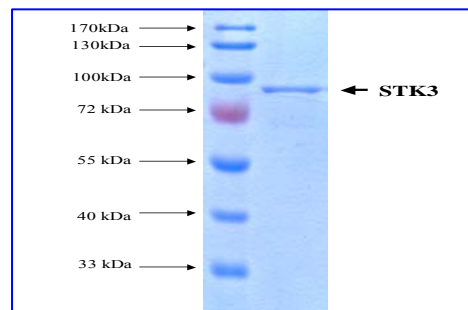


Fig. 2 STK3 protein gel

Purity assessment

1 µg of STK3 protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >90% purity of the STK3 product, and the band was at ~87 kDa (Fig. 2).

Product Description

Recombinant full-length human STK3 containing N-terminal GST tag was expressed by baculovirus in Sf9 insect cells.

The gene accession number is BC010640.

This material is sold for research purposes only.

Specific Activity

277 nmol phosphate incorporated into MBP per minute per mg protein at 30°C for 15 minutes using a final concentration of 50 µM ATP (0.83 µCi/assay).

Formulation

Recombinant protein in storage buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol).

Storage and Stability

Store product frozen at or below -70°C. Stable for 1 year at -70°C as undiluted stock. Aliquot to avoid repeated thawing and freezing.

Scientific Background

Creasy identified a cDNA encoding STK3, which they called MST2. Sequence analysis indicated that the deduced 491-amino acid STK3 protein contains an N-terminal catalytic domain characteristic of STKs (1). Taylor also isolated cDNAs encoding STK3 (p63) and STK4 (p61) respectively. STK3 and STK4 share 94% amino acid identity in the catalytic domain and 78% identity overall (2). The yeast 'sterile 20' (Ste20) kinase acts upstream of the mitogen-activated protein kinase (MAPK) cascade that is activated under a variety of stress conditions. O'Neill used proteomic analysis of RAF1 signaling complexes to show that RAF1 counteracts apoptosis by suppressing the activation of mammalian sterile 20-like kinase (MST2) (3). MST2 was identified as a kinase that is activated by the proapoptotic agents staurosporine and FAS ligand (2;4). STK3 activation presumably allows cells to resist unfavorable environmental conditions.

References

1. Creasy, C. L.; Chernoff, J.: Cloning and characterization of a member of the MST subfamily of Ste20-like kinases. *Gene* 167: 303-306, 1995.
2. Taylor, L. K.; Wang, H.-C. R.; Erikson, R. L.: Newly identified stress-responsive protein kinases, Krs-1 and Krs-2. *Proc. Nat. Acad. Sci.* 93: 10099-10104, 1996.
3. O'Neill, E.; Rushworth, L.; Baccarini, M.; Kolch, W.: Role of the kinase MST2 in suppression of apoptosis by the proto-oncogene product Raf-1. *Science* 306: 2267-2270, 2004.
4. Lee, K.-K.; Ohyama, T.; Yajima, N.; Tsubuki, S.; Yonehara, S.: MST, a physiological caspase substrate, highly sensitizes apoptosis both upstream and downstream of caspase activation. *J. Biol. Chem.* 276: 19276-19285, 2001.