p70 S6 kinase (T412E) [6His-tagged]

Kinase

Alternate Names: Ribosomal protein S6 kinase 1, P70S6K1

Cat. No. **66-0039-050**
Lot. No. **30318**

Quantity: 50 µg
Storage: -70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS Page 1 of 2

Background

Protein ubiquitylation and protein phosphorylation are the two major mechanisms that regulate the functions of proteins in eukaryotic cells. However, these different posttranslational modifications do not operate independently of one another, but are frequently interlinked to enable biological processes to be controlled in a more complex and sophisticated manner. Studying how protein phosphorylation events control the ubiquitin system and how ubiquitylation regulates protein phosphorylation has become a focal point of the study of cell regulation and human disease. Cloning of p70 S6 kinase was first described by Grove *et al.* (1991). An example of such interplay between phosphorylation and ubiquitylation has been highlighted in a recent study showing that p70 S6 kinase phosphorylates TRIB2 (Tribbles homolog 2) enabling this protein to ubiquitylate the E3 ligase Smurf1 (Smad ubiquitination regulatory factor 1). It is thought that impaired phosphorylation and ubiquitylation by p70 S6 kinase and Smurf1 respectively increase the protein stability of TRIB2 in liver cancer and thus may be helpful in the development of diagnosis and treatment strategies against this malignant disease (Wang *et al.*, 2013).

References:

Alessi DR, Kozlowski MT, Weng QP, Morrice N, Avruch J (1998) 3-Phosphoinositide-dependent protein kinase 1 (PDK1) phosphorylates and activates the p70 S6 kinase *in vivo* and *in vitro*. *Curr Biol* **15**, 69-81.

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Physical Characteristics

Species: rat

Source: baculovirus expression vector system

Quantity: 50 µg

Concentration: 1.1 mg/ml

Formulation: 50 mM Tris/HCl pH7.5, 0.1 mM EGTA, 150 mM NaCl, 0.1% ß-Mercaptoethanol, 270 mM sucrose, 0.03% Brij-35, 1 mM Benzamidine, 0.2 mM PMSF

Molecular Weight: ~48.6 kDa

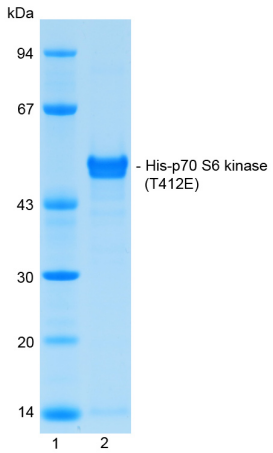
Purity: >95% by InstantBlue™ SDS-PAGE

Stability/Storage: 12 months at -70˚C; aliquot as required

Protein Sequence: Please see page 2

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 2.5 µg His-p70 S6 kinase (T412E)



Protein Identification:
Confirmed by mass spectrometry.

Activity Assay:
The specific activity of His-p70 S6 kinase (T412E) was determined using the method described by Hastie *et al.* (2006) with the enzyme being assayed at several concentrations. His-p70 S6 kinase (T412E) was incubated for 10 minutes at 30˚C in kinase reaction buffer in the presence of CROSStide substrate (30 µM) and [γ-32P] ATP (100 µM). Duplicate reactions were stopped by spotting the assay mixture onto Whatman P81 paper – capturing the phosphorylated substrate.The radioactivity incorporated was measured on a scintillation counter and the enzyme's mean specific activity was calculated.

His-p70 S6 kinase (T412E) specific activity:
154.2 Units/mg (169.6 Units/ml)

1 Unit = 1 nmole of phosphate incorporated into the substrate in 1 minute

Substrate: CROSStide (GRPRTSSFAEG)

www.ubiquigent.com
Dundee, Scotland, UK

ORDERS / SALES SUPPORT
International: +1-617-245-0003
US Toll-Free: 1-888-4E1E2E3 (1-888-431-3233)
Email: sales.support@ubiquigent.com

UK HQ and TECHNICAL SUPPORT
International: +44 (0) 1382 381147 (9AM-5PM UTC)
US/Canada: +1-617-245-0020 (9AM-5PM UTC)
Email: tech.support@ubiquigent.com

Email **services@ubiquigent.com** for enquiries regarding compound profiling and/or custom assay development services.

Lot-specific COA version tracker: v1.0.0

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Background

Continued from page 1

Grove JR, Banerjee P, Balasubramanyam A, Coffer PJ, Price DJ, Avruch J, *et al.* (1991) Cloning and expression of two human p70 S6 kinase polypeptides differing only at their amino termini. *Mol Cell Biol* **11**, 5541-5550.

Hastie CJ, McLauchlan HJ, Cohen P (2006) Assay of protein kinases using radiolabeled ATP: a protocol. *Nat Protoc* **1**, 968-71.

Wang J, Zhang Y, Weng W, Qiao Y, Ma L, Xiao W, *et al.* (2013) Impaired phosphorylation and ubiquitination by p70 S6 kinase (p70S6K) and Smad ubiquitination regulatory factor 1 (Smurf1) promote tribbles homolog 2 (TRIB2) stability and carcinogenic property in liver cancer. *J Biol Chem* **288**, 33667-33681.

Physical Characteristics

Continued from page 1

Protein Sequence:

MHHHHHHMRRRRRRDGFYPAPDFRHREAE
DMAGVFDIDLDQPEDAGSEDELEEGGQLNESM
DHGGVGPYELGMEHCEKFEISETSVNRGPE
KIRPECFELLRVLGKGGYGKVFQVRKVTGANT
GKIFAMKVLKKAMIVRNAKDTAHTKAERNI
LEEVKHPFIVDLIYAFQTGGKLYLILEYLSG
GELFMQLEREGIFMEDTACFYLAEISMALGHL
HQKGIIYRDLKPENIMLNHQGHVKLTDFGLCK
ESIHDGTVTHTFCGTIEYMAPEILMRSGHN
RAVDWWSLGALMYDMLTGAPPFTGENRKKTID
KILKCKLNLPPYLTQEARDLLKKLLKRNAASR
LGAGPGDAGEVQAHPFFRHINWEELLARKVE
PPFKPLLQSEEDVSQFDSKFTRQTPVDSP
DDSTLSESANQVFLGFEYVAPSVLES

Tag (**bold text**): N-terminal 6His
Protease cleavage site: none
p70 S6 Kinase (regular text): Start ***bold italics*** (amino acid residues 1-421).
p70 S6 kinase has a T412E mutation to mimic the activation of the enzyme through phosphorylation of Thr412 by PDK2 (Alessi *et al.*, 1998).
Accession number: P67999

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Dundee, Scotland, UK

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