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

Kinase

Alternate Names: Ribosomal protein S6 kinase 1, P70S6K1

Cat. No.	66-0039-050	Quantity:	50 μg
Lot. No.	30318	Storage:	-70°C
FOR RESEARCH USE ONLY		NOT FOR USE IN HUMANS	



CERTIFICATE OF ANALYSIS Page 1 of 2

Protein Sequence: Please see page 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 requlation 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.

Continued on page 2



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

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 [v-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)

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.

© Ubiquigent 2014. Unless otherwise noted, Ubiquigent, Ubiquigent logo and all other trademarks are the property of Ubiquigent, Ltd.

Limited Terms of Use: For research use only. Not for use in humans or for diagnostics. Not for distribution or resale in any form, modification or derivative OR for use in providing services to a third party (e.g. screening or profiling) without the written permission of Ubiquigent, Ltd.

Lot-specific COA version tracker: v1.0.0

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

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

Kinase

Alternate Names: 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 2 of 2

Background

Physical Characteristics

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 (Smuf1) promote tribbles homolog 2 (TRIB2) stability and carcinogenic property in liver cancer. J Biol Chem **288**, 33667-33681. 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



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. © Ubiquigent 2014. Unless otherwise noted, Ubiquigent, Ubiquigent logo and all other trademarks are the property of Ubiquigent, Ltd.

Limited Terms of Use: For research use only. Not for use in humans or for diagnostics. Not for distribution or resale in any form, modification or derivative QR for use in providing services to a third party (e.g. screening or profiling) without the written permission of Ubiquigent, Ltd.

Lot-specific COA version tracker: v1.0.0