BRSK2 [6His-tagged]

Kinase

Alternate Names: BR Serine/Threonine Kinase 2; SAD1; PEN11B

Cat. No.	**66-0001-050**	Quantity:	50 µg
Lot. No.	**2138**	Storage:	-70˚C

FOR RESEARCH USE ONLY NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS Page 1 of 2

Background by Sir Philip Cohen

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 human Brain Specific Kinase 2 (BRSK2) was first described by Miura *et al.* (1998). BRSK2 is a member of the subfamily of protein kinases that include the AMP-activated protein kinase (AMPK) and, like AMPK itself, is activated by the tumour suppressor kinase LKB1 (Lizcano *et al.*, 2004). As implied by its name BRSK2 is expressed in the brain where it plays an essential role in controlling neuronal cell polarisation. BRSK2 contains a ubiquitin-like domain adjacent to the kinase catalytic domain (Al-Hakim *et al.*, 2008).

References:

Al-Hakim AK, Zagorska A, Chapman L, Deak M, Peggie M, Alessi DR (2008) Control of AMPK-related kinases by USP9X and atypical Lys(29)/Lys(33)-linked polyubiquitin chains. *Biochem J* **411**, 249-60.

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

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Physical Characteristics

Species: human

Source: Sf21 insect cell-baculovirus expression

Quantity: 50 µg

Concentration: 1 mg/ml

Formulation: 50 mM Tris/HCl pH 7.5, 0.1 mM EGTA, 150 mM NaCl, 270 mM sucrose, 0.03% Brij, 0.1% β-Mercaptoethanol, 1 mM Benzamidine, 0.2 mM PMSF

Molecular Weight: ~78.3 kDa

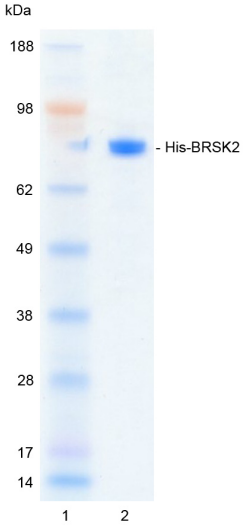
Purity: >95% by InstantBlue™ SDS-PAGE

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: 1 µg His-BRSK2



Protein Identification:
Confirmed by mass spectrometry.

Activity Assay:
The specific activity of His-BRSK2 was determined using the method described by Hastie *et al.* (2006) with the enzyme being assayed at several concentrations. His-BRSK2 was incubated for 10 minutes at 30˚C in kinase reaction buffer in the presence of CHKtide substrate (300µM) and [γ-^{32}P]ATP (100µM). 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-BRSK2 specific activity:
333.7 Units/mg (333.7 Units/ml)

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

Substrate: CHKtide (KKKVSRSGLYRSPSMPENLNRPR)

www.ubiquigent.com
Dundee, Scotland, UK

ORDERS / SALES SUPPORT
International: +1-617-245-0003
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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 by Sir Philip Cohen

Continued from page 1

Miura K, Masuzaki H, Ishimaru T, Niikawa N, Jinno Y (1998) A HhaI/BstUI polymorphism in a novel gene at human chromosome 11p15.5. *J Hum Genet* **90**, 283-4.

Lizcano JM, Goransson O, et al. (2004) LKB1 is a master kinase that activates 13 kinases of the AMPK subfamily, including MARK/PAR-1. *EMBO J* **23**, 833-43.

Background kindly written by:

Sir Philip Cohen FRS, FRSE
University of Dundee

Director of the Medical Research Council Protein Phosphorylation Unit (1990-2012)

Director of the Scottish Institute for Cell Signalling incorporating the Protein Ubiquitylation Unit (2008-2012)

Co-Director of the Division of Signal Transduction Therapy (1998-2012)

Deputy Director of the Division of Signal Transduction Therapy (from July 2012)

Professor Cohen's research group is studying the interplay between protein phosphorylation and protein ubiquitylation in the regulation of innate immunity.

Physical Characteristics

Continued from page 1

Protein Sequence:

MSYYHHHHHHDYDIPTTENLYFQGAMGS***T***
STGKDGGAQHAQYVGPYRLEKTLGKGQT
GLVKLGVHCVTCQKVAIKIVNREKLS
ESVLMKVEREIAILKLIEHPHVLKLHD
VYENKKYLYLVLEHVSGGELFDYLVK
KGRLTPKEARKFFRQIISALDFCHSH
SICHRDLKPENLLLDEKNNIRIADFG
MASLQVGDSLLETSCGSPHYACPEVIR
GEKYDGRKADVWSCGVILFALLVGALPFD
DDNLRQLLEKVKRGVFHMPHFIPPDC
QSLLRGMIEVDAARRLTLEHIQKHIWYIG
GKNEPEPEQPIPRKVQIRSLPSLEDIDP
DVLDSMHSLGCFRDRNKLLQDLLSEEEN
QEKMIYFLLLDRKERYPSQEDEDLPPR
NEIDPPRKRVDSPMLNRHGKRRPERKS
MEVLSVTDGGSPVPARRAIEMAQH
GQRSRSISGASSGLSTSPLSSPRVT
PHPSPRGSPLPTPKGTPVHTPKESPAGT
PNPTPPSSPSVGGVPWRARLNSIKNS
FLGSPRFHRRKLQVPTPEEMSNLTPESS
PELAKKSWFGNFISLEKEEQIFVVIKDK
PLSSIKADIVHAFLSIPSLSHSVISQTS
FRAEYKATGGPAVFQKPVKFQVDI
TYTEGGEAQKENGIYSVTFTLLSGPSR
RFKRVVETIQAQLLSTHDPPAAQHLSEPP
PPAPGLSWGAGLKGQKVATSYESSL

Tag (**bold text**): N-terminal His
Protease cleavage site: TEV (ENLYF▼QG)
BRSK2 (regular text): Start ***bold italics*** (amino acid residues 2-674)
Accession number: AAP97725.1

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US/Canada: +1-617-245-0020 (9AM-5PM UTC)
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