

Certificate of Analysis

BrSK1, active

(Recombinant enzyme expressed in Sf21 insect cells.)

Item # 14-675, 14-675-K, 14-675M

Parent Lot # 33242U

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: N-terminal 6His-tagged, recombinant, human BrSK1 amino acids 2-end, expressed by baculovirus in Sf21 cells. Purified using Ni²⁺/NTA agarose. Purity 85% by SDS-PAGE and Coomassie blue staining. MW = 88.4kDa.

Specific Activity (Parent lot# 33242U): 310U/mg, where one unit of BrSK1, active activity is defined as 1nmol phosphate incorporated into 100µM CHKtide (KKKVSRSGLYRSPSPENLNRPR) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 3.656mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

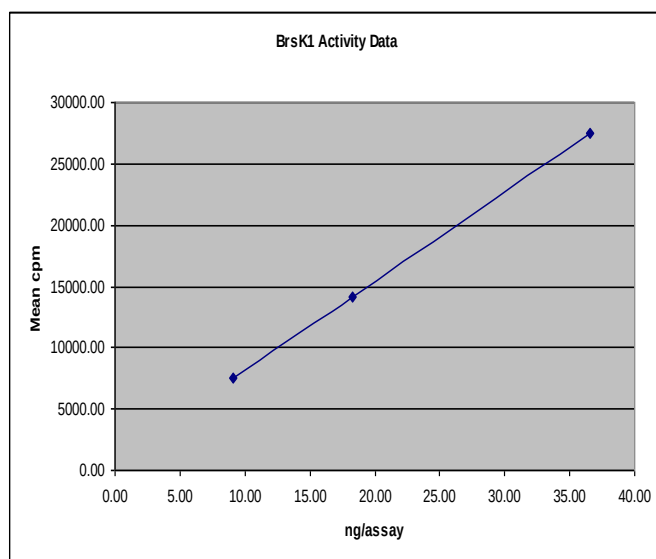
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

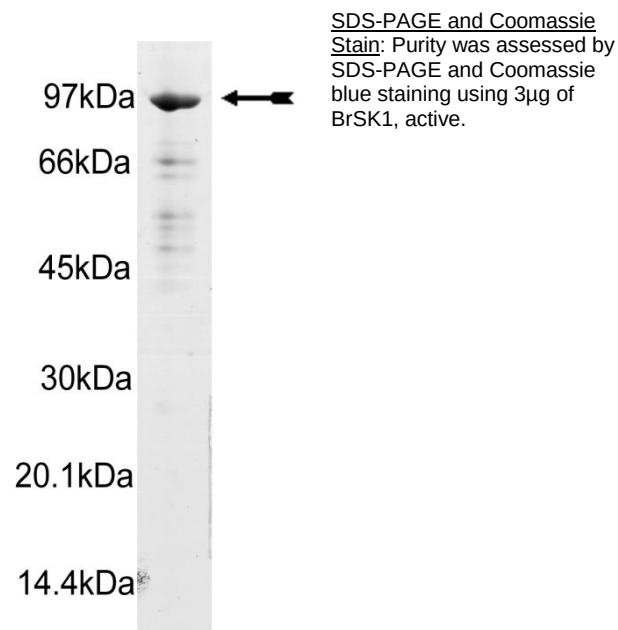
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 9–36ng of this lot of enzyme phosphorylated 100µM CHKtide in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed identity as BrSK1 with the translated native sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS-NaOH pH7.0, 1mM EDTA.
2. **CHKtide (KKKVSRSGLYRSPSPENLNRPR):** Use at a final assay concentration of 100 μ M. Prepare a 1mM stock. Use 2.5 μ l of stock per assay point.
3. **BrSK1, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 9–36ng per assay point.
4. **[γ -³³P]ATP:** 2.5 x magnesium acetate/[γ -³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 5 μ l of 5 x reaction buffer per assay to wells
2. Add 2.5 μ l of **CHKtide**.
3. Add **2.5 μ l (9–36ng) BrSK1, active**.
4. Add 5 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ l aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1 μ l of 30% phosphoric acid.

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BrSK1 Sequence Information

<u>Protein</u>	Human BrSK1
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	S29 of the recombinant sequence is equivalent to S2 of native human BrSK1
<u>Accession number</u>	GenBank NM_032430

Recombinant BrSK1 amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMGSSS GAKEGGGGSP AYHLPHPHPH PPQHAQYVGP
61 YRLEKTLGKG QTGLVKLG VH CITGQKVAIK IVNREKLSES VLMKVEREIA ILKLIEHPHV
121 LKLHDVYENK KYLYLVLEHV SGGELFDYLV KKGR LTPKEA RKFFRQIVSA LDFCHSYSIC
181 HRDLKPENLL LDEKNNIRIA DFGMASLQVG DS LLETSCGS PHYACPEVIK GEKYDGRRAD
241 MWSCGVILFA LLVGALPFDD DNLRQLLEKV KRGV FHMHPHF IPPDCQSLLR GMIEVEPEKR
301 LSLEQIQKHP WYLGGKHEPD PCLEPAPGRR VAMRSLPSNG ELDPDVLESM ASLGCFRDRE
361 RLHREL RSEE ENQEKMIYYL LLDRKERYPS CEDQDLPPRN DVDPPRKRVD SPMLSRHGKR
421 RPERKSMEVL SITDAGGGGS PVPTRRALEM AQHSQRSRSV SGASTGLSSS PLSSPRSPVF
481 SFSPEPGAGD EARGGGSP TS KTQTLPSRGP RGGGAGEQPP PPSARSTPLP GPPGSPRSSG
541 GTPLHSPLHT PRASPTGTPG TTPPPSPGGG VGGA AWR SRL NSIRNSFLGS PRFHRRKMQV
601 PTAEEMSSLT PESSPELAKR SWFGNFISLD KEEQIFLV LK DKPLSSIKAD IVHAF LSIPS
661 LSHSVLSQTS FRAEYKASGG PSVFQKPVR F QVDISSSEGP EPSPRRDGSG GGGIYSVTFT
721 LISGPSRRFK RVETIQ AQL LSTHDQPSVQ ALADEKNGAQ TRPAGAPPRS LQPPPGRPDP
781 ELSSSPRRGP PKDKLLATN GTPLP
  
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Recombinant BrSK1 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
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1561 cccccagtg cccgtccac acccctgcc ggccccccag gctccccgcg ctctctggc
  
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1681 acaacaccac ccccagccc cggcgggtggc gtcgggggag ccgcctggag gaggcgtctc
1741 aactccatcc gcaacagctt cctgggctcc cctcgctttc accggcgcaa gatgcaggtc
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2401 gggacccctc tgccctga

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