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Certificate of Analysis

BrSK2, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-655, 14-655-K, 14-655M
Parent Lot # 28254U

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 BrSK2, amino acids 2–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose. Purity 67% by SDS-PAGE and Coomassie blue staining. MW = 78.5kDa.

Specific Activity (Parent lot# 28254U): 197U/mg, where one unit of BrSK2 activity is defined as 1nmol phosphate incorporated into 500µM (KKLNRTLSFAEPG) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.77mg/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.

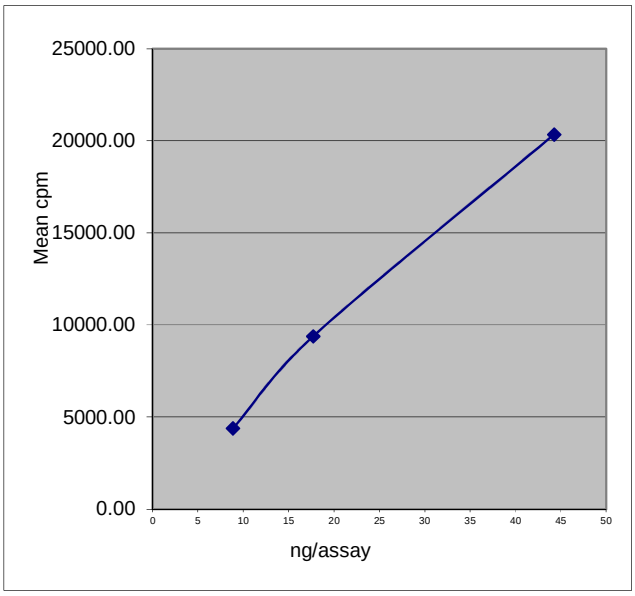
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

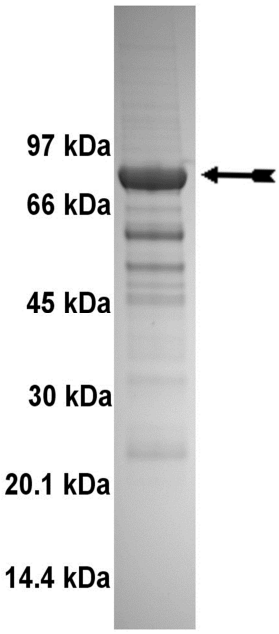
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 8–44ng of this lot of enzyme phosphorylated 500µM (KKLNRTLSFAEPG) 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 BrSK2 with the translated native sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of BrSK2, active.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS-NaOH pH7.0, 1mM EDTA.
2. **(KKLNRTLSFAEPG):** Use at a final concentration of 500µM. Make a 5mM stock. Add 2.5µl of stock per assay point.
3. **BrSK2, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 8–44ng per assay point.
4. **[γ-^{33}P]ATP:** 2.5 x magnesium acetate/[γ-^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-^{33}P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of 500µM **(KKLNRTLSFAEPG)**.
3. Add **2.5µl (8–44ng) BrSK2, active**.
4. Add 5µl of dH_2O.
5. Add 10µl of diluted [γ-^{33}P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl 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 1µl of 30% phosphoric acid.

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BrSK2 Sequence Information

Protein Human BrSK2

Tags *N*-terminal 6His

Native sequence T29 of the recombinant protein is equivalent to T2 of human BrSK2

Accession number GenBank AF533878. The recombinant protein contains the amino acid substitution S251I with respect to AF533878. This conflict is reported in GenBank AF020086.

Recombinant BrSK2 amino acid sequence:

```
  1 MSYYHHHHHH DYDIPTTENL YFQGAMGSTS TGKDGGAQHA QYVGPYRLEK TLGKGQTGLV
 61 KLGVHCVTCQ KVAIKIVNRE KLSESVLMKV EREIAILKLI EHPHVLKLHD VYENKKYLYL
121 VLEHVSGGEL FDYLVKKGRL TPKEARKFFR QIISALDFCH SHSICHRDLK PENLLLDEKN
181 NIRIADFGMA SLQVGDSLLE TSCGSPHYAC PEVIRGEKYD GRKADVWSCG VILFALLVGA
241 LPFDDDNLRQ LLEKVKRGVF HMPHFIPPDC QSLLRGMIEV DAARRLTLEH IQKHIWYIGG
301 KNEPEPEQPI PRKVQIRSLP SLEDIDPDVL DSMHSLGCFR DRNKLLQDLL SEEENQEKMI
361 YFLLLDRKER YPSQEDEDLP PRNEIDPPRK RVDSPMLNRH GKRRPERKSM EVLSVTDGGS
421 PVPARRAIEM AQHGQRSRSI SGASSGLSTS PLSSPRVTPH PSPRGSPLPT PKGTPVHTPK
481 ESPAGTPNPT PPSSPSVGGV PWRARLNSIK NSFLGSPRFH RRKLQVPTPE EMSNLTPESS
541 PELAKKSWFG NFISLEKEEQ IFVVIKDKPL SSIKADIVHA FLSIPSLSHS VISQTSFRAE
601 YKATGGPAVF QKPVKFQVDI TYTEGGEAQK ENGIYSVTFT LLSGPSRRFK RVVETIQAQL
661 LSTHDPPAAQ HLSEPPPPAP GLSWGAGLKG QKVATSYESS L
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Recombinant BrSK2 nucleotide sequence:

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   1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
  61 tattttcagg gcgccatggg atccacatcg acggggaagg acggcggcgc gcagcacgcg
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 961 agcctggagg acatcgaccc cgacgtgctg gacagcatgc actcactggg ctgcttccga
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1681 atcttcgtgg tcatcaaaga caaacctctg agctccatca aggctgacat cgtgcacgcc
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1741 ttcctgtcga ttcccagtct cagccacagc gtcatctccc aaacgagctt ccgggccgag
1801 tacaaggcca cgggggggcc agccgtgttc cagaagccgg tcaagttcca ggttgatatc
1861 acctacacgg agggtgggga ggcgcagaag gagaacggca tctactccgt caccttcacc
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1981 ctgagcacac acgacccgcc tgcggcccag cacttgtcag aaccccccc accagcgcca
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2101 ctctga
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