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# Certificate of Analysis

## PKB beta (S474D)

**(Recombinant enzyme expressed in Sf21 insect cells)**
**Item # 14-339, 14-339-K, 14-339M**
**Parent Lot # D8JN055U**

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 PKB beta residues 120–end containing the S474D mutation. Expressed by baculovirus in Sf21 insect cells. Purified using $Ni^{2+}$/NTA agarose, activated by PDK1, and repurified by heparin-Sepharose. Purity 81.1% by SDS-PAGE and Coomassie blue staining. MW = 42.8kDa.

**Specific Activity (Parent lot# D8JN055U):** 276U/mg, where one unit of PKB beta activity is defined as 1nmol phosphate incorporated into 30µM modified crosstide (GRPRTSSFAEGKK) per minute at 30°C with a final ATP concentration of 100µM.

**Formulation: 0.662mg/ml** of enzyme in 50mM Tris/HCl pH7.5, 270mM sucrose, 150mM NaCl, 1mM EGTA, 1mM benzamidine, 0.2mM PMSF, 0.1 % 2-mercaptoethanol, 0.03 % Brij-35. Frozen solution.

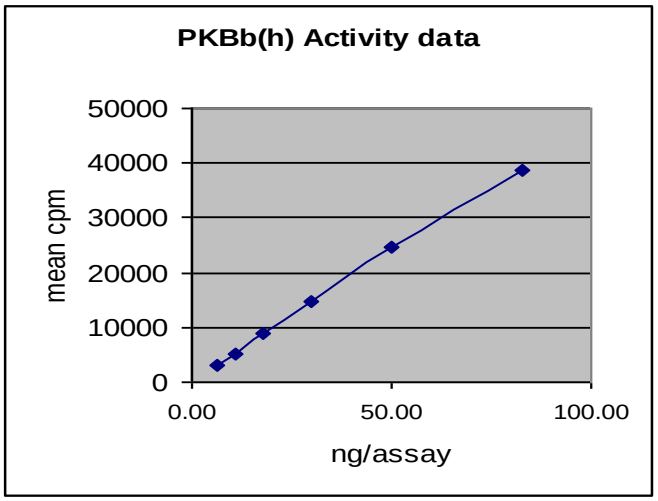
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**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.

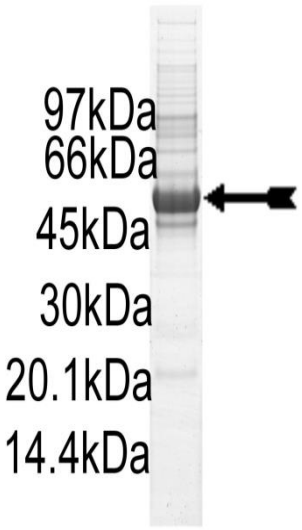
**FOR IN VITRO RESEARCH USE ONLY**
**NOT FOR USE IN HUMANS OR ANIMALS**

## Quality Control Testing

Kinase Assay: 6.6–82.8ng of this lot of enzyme phosphorylated 30µM modified crosstide 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 PKB beta with the translated sequence listed on page three.




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

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**Kinase Assay Protocol**

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **Modified Crosstide (GRPRTSSFAEGKK):** Use at a final assay concentration of 30µM. Prepare a 300µM stock and add 2.5µl of stock per assay point.
3. **PKB beta, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 6.6–82.8ng 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 well.
2. Add 2.5µl of **modified crosstide**.
3. Add **2.5µl (6.6–82.8ng) PKB beta, 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 10 µl aliquot onto the appropriate area of a **P30 Filtermat.**
9. Wash the filtermat three times for 5 minutes with 50mM 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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## PKB beta Sequence Information

**Protein** human PKB beta (S474D)

**Tags** *N*-terminal 6His

**Native sequence** M8 of the recombinant protein is equivalent to M120 of human PKB beta

**Accession number** EMBL M95936. The recombinant protein contains an amino acid substitution, S474D, with reference to the native sequence. This mimics the phosphorylation of S474.

***Recombinant PKB beta amino acid sequence:***

```
  1 MHHHHHHMDY KCGSPSDSST TEEMEVAVSK ARAKVTMNDF DYLKLLGKGT FGKVILVREK
 61 ATGRYYAMKI LRKEVIIAKD EVAHTVTESR VLQNTRHPFL TALKYAFQTH DRLCFVMEYA
121 NGGELFFHLS RERVFTEERA RFYGAEIVSA LEYLHSRDVV YRDIKLENLM LDKDGHIKIT
181 DFGLCKEGIS DGATMKTFCG TPEYLAPEVL EDNDYGRAVD WWGLGVVMYE MMCGRLPFYN
241 QDHERLFELI LMEEIRFPRT LSPEAKSLLA GLLKKDPKQR LGGGPSDAKE VMEHRFFLSI
301 NWQDVVQKKL LPPFKPQVTS EVDTRYFDDE FTAQSITITP PDRYDSLGLL ELDQRTHFPQ
361 FDYSASIRE
```

***Recombinant PKB beta nucleotide sequence:***

```
   1 atgcatcatc accatcacca tatggactac aagtgtggct cccccagtga ctcctccacg
  61 actgaggaga tggaagtggc ggtcagcaag gcacgggcta aagtgaccat gaatgacttc
 121 gactatctca aactccttgg caagggaacc tttggcaaag tcatcctggt gcgggagaag
 181 gccactggcc gctactacgc catgaagatc ctgcggaagg aagtcatcat tgccaaggat
 241 gaagtcgctc acacagtcac cgagagccgg gtcctccaga acaccaggca cccgttcctc
 301 actgcgctga agtatgcctt ccagacccac gaccgcctgt gctttgtgat ggagtatgcc
 361 aacgggggtg agctgttctt ccacctgtcc cgggagcgtg tcttcacaga ggagcgggcc
 421 cggttttatg gtgcagagat tgtctcggct cttgagtact tgcactcgcg ggacgtggta
 481 taccgcgaca tcaagctgga aaacctcatg ctggacaaag atggccacat caagatcact
 541 gactttggcc tctgcaaaga gggcatcagt gacggggcca ccatgaaaac cttctgtggg
 601 accccggagt acctggcgcc tgaggtgctg gaggacaatg actatggccg ggccgtggac
 661 tggtgggggc tgggtgtggt catgtacgag atgatgtgcg gccgcctgcc cttctacaac
 721 caggaccacg agcgcctctt cgagctcatc ctcatggaag agatccgctt cccgcgcacg
 781 ctcagccccg aggccaagtc cctgcttgct gggctgctta agaaggaccc caagcagagg
 841 cttggtgggg ggcccagcga tgccaaggag gtcatggagc acaggttctt cctcagcatc
 901 aactggcagg acgtggtcca gaagaagctc ctgccaccct tcaaacctca ggtcacgtcc
 961 gaggtcgaca caaggtactt cgatgatgaa tttaccgccc agtccatcac aatcacaccc
1021 cctgaccgct atgacagcct gggcttactg gagctggacc agcggaccca cttcccccag
1081 ttcgactact cggccagcat ccgcgagtga
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Reviewed and approved by site quality representative.