eurofins | Discovery Services

Certificate of Analysis

TBK1, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-628, 14-628-K, 14-628M
Parent Lot # 2284433

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, full length, human TBK1, expressed by baculovirus in Sf21 insect cells. Purified using immobilised metal affinity chromatography. Purity 85.7% by SDS-PAGE and Coomassie blue staining.
MW = 85.7kDa.

Specific Activity (Parent lot# 2284433): 2215U/mg, where one unit of TBK1 activity is defined as 1nmol phosphate incorporated into 200µM (KRRRALS(p)VASLPGL) (CK1tide) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.364mg/ml of enzyme in 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.

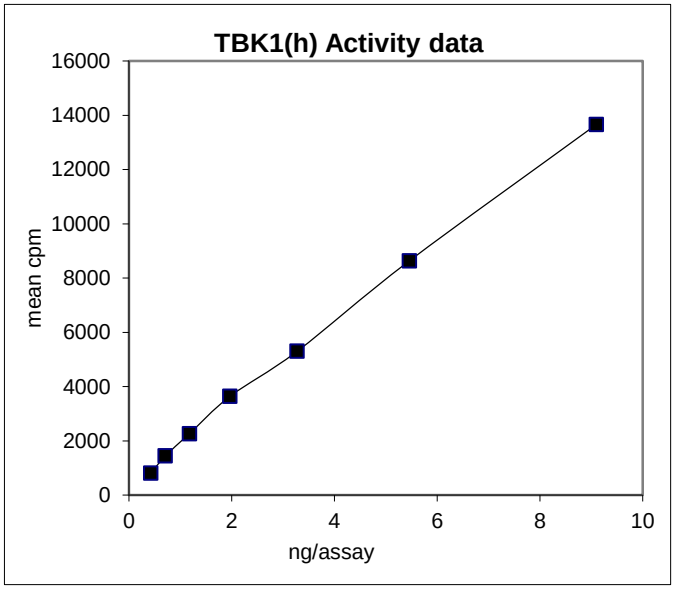
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 6 months from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

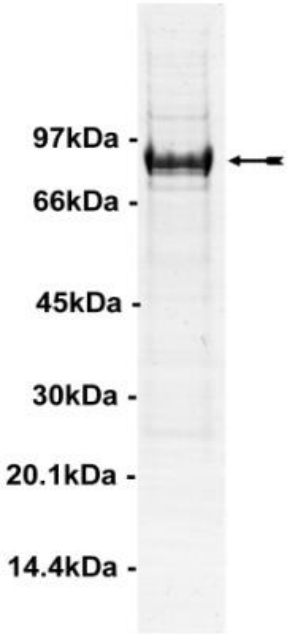
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 0.4–9.1ng of this lot of enzyme phosphorylated 200µM (KRRRALS(p)VASLPGL) 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 product identity as TBK1 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 active TBK1

Eurofins Pharma Discovery Services UK Limited
Gemini Crescent
Dundee Technology Park
DUNDEE
DD2 1SW United Kingdom
T | +44 (0)1382 561600
F | +44 (0)1382 561601
www.eurofins.com/pharmadiscovery

Certificate of Analysis

Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **(KRRRALS(p)VASLPGL):** Use at a final assay concentration of 200µM. Prepare a 2mM stock. Use 2.5 µl of stock per assay point.
3. **TBK1, active:** Dilute with 20mM MOPS/NaOH pH 7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.4–9.1ng 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 10 x reaction buffer per assay to wells.
2. Add 2.5µl of **(KRRRALS(p)VASLPGL)**.
3. Add **2.5µl (0.4–9.1ng) TBK1, 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.

eurofins | Discovery Services

Certificate of Analysis

TBK1 Sequence Information

Protein	Human TBK1
Tags	*N*-terminal 6His
Native sequence	M16 of the recombinant sequence is equivalent to M1 of native human TBK1
Accession number	GenBank NM_013254

Recombinant TBK1 amino acid sequence:

```
  1 MHHHHHHEFK GLRRQMQSTS NHLWLLSDIL GQGATANVFR GRHKKTGDLF AIKVFNNISF
 61 LRPVDVQMRE FEVLKKLNHK NIVKLFAIEE ETTTRHKVLI MEFCPCGSLY TVLEEPSNAY
121 GLPESEFLIV LRDVVGGMNH LRENGIVHRD IKPGNIMRVI GEDGQSVYKL TDFGAARELE
181 DDEQFVSLYG TEEYLHPDMY ERAVLRKDHQ KKYGATVDLW SIGVTFYHAA TGSLPFRPFE
241 GPRRNKEVMY KIITGKPSGA ISGVQKAENG PIDWSGDMPV SCSLSRGLQV LLTPVLANIL
301 EADQEKCWGF DQFFAETSDI LHRMVIHVFS LQQMTAHKIY IHSYNTATIF HELVYKQTKI
361 ISSNQELIYE GRRLVLEPGR LAQHFPKTTE ENPIFVVSRE PLNTIGLIYE KISLPKVHPR
421 YDLDGDASMA KAITGVVCYA CRIASTLLLY QELMRKGIRW LIELIKDDYN ETVHKKTEVV
481 ITLDFCIRNI EKTVKVYEKL MKINLEAAEL GEISDIHTKL LRLSSSQGTI ETSLQDIDSR
541 LSPGGSLADA WAHQEGTHPK DRNVEKLQVL LNCMTEIYYQ FKKDKAERRL AYNEEQIHKF
601 DKQKLYYHAT KAMTHFTDEC VKKYEAFLNK SEEWIRKMLH LRKQLLSLTN QCFDIEEEVS
661 KYQEYTNELQ ETLPQKMFTA SSGIKHTMTP IYPSSNTLVE MTLGMKKLKE EMEGVVKELA
721 ENNHILERFG SLTMDGGLRN VDCL
```

Recombinant TBK1 nucleotide sequence:

```
   1 atgcatcatc accatcacca tgaattcaaa ggcctacgtc gacaaatgca gagcacttct
  61 aatcatctgt ggcttttatc tgatatttta ggccaaggag ctactgcaaa tgtctttcgt
 121 ggaagacata agaaaactgg tgatttattt gctatcaaag tatttaataa cataagcttc
 181 cttcgtccag tggatgttca aatgagagaa tttgaagtgt tgaaaaaact caatcacaaa
 241 aatattgtca aattatttgc tattgaagag gagacaacaa caagacataa agtacttatt
 301 atggaatttt gtccatgtgg gagtttatac actgttttag aagaaccttc taatgcctat
 361 ggactaccag aatctgaatt cttaattgtt ttgcgagatg tggtgggtgg aatgaatcat
 421 ctacgagaga atggtatagt gcaccgtgat atcaagccag gaaatatcat gcgtgttata
 481 ggggaagatg gacagtctgt gtacaaactc acagattttg gtgcagctag agaattagaa
 541 gatgatgagc agtttgtttc tctgtatggc acagaagaat atttgcaccc tgatatgtat
 601 gagagagcag tgctaagaaa agatcatcag aagaaatatg gagcaacagt tgatctttgg
 661 agcattgggg taacatttta ccatgcagct actggatcac tgccatttag acccttttgaa
 721 gggcctcgta ggaataaaga agtgatgtat aaaataatta caggaaagcc ttctggtgca
 781 atatctggag tacagaaagc agaaaatgga ccaattgact ggagtggaga catgcctgtt
 841 tcttgcagtc tttctcgggg tcttcaggtt ctacttaccc ctgttcttgc aaacatcctt
 901 gaagcagatc aggaaaagtg ttggggtttt gaccagtttt ttgcagaaac tagtgatata
 961 cttcaccgaa tggtaattca tgtttttcg ctacaacaaa tgacagctca taagatttat
1021 attcatagct ataatactgc tactatattt catgaactgg tatataaaca aaccaaaatt
1081 atttcttcaa atcaagaact tatctacgaa gggcgacgct tagtcttaga acctggaagg
1141 ctggcacaac atttccctaa aactactgag gaaaacccta tatttgtagt aagccgggaa
1201 cctctgaata ccataggatt aatatatgaa aaaatttccc tccctaaagt acatccacgt
1261 tatgatttag acggggatgc tagcatggct aaggcaataa caggggttgt gtgttatgcc
1321 tgcagaattg ccagtacctt actgctttat caggaattaa tgcgaaaggg gatacgatgg
1381 ctgattgaat taattaaaga tgattacaat gaaactgttc acaaaaagac agaagttgtg
1441 atcacattgg atttctgtat cagaaacatt gaaaaaactg tgaaagtata tgaaaagttg
1501 atgaagatca acctggaagc ggcagagtta ggtgaaattt cagacataca caccaaattg
1561 ttgagacttt ccagttctca gggaacaata gaaaccagtc ttcaggatat cgacagcaga
```

Certificate of Analysis

```
1621 ttatctccag gtggatcact ggcagacgca tgggcacatc aagaaggcac tcatccgaaa
1681 gacagaaatg tagaaaaact acaagtcctg ttaaattgca tgacagagat ttactatcag
1741 ttcaaaaaag acaaagcaga acgtagatta gcttataatg aagaacaaat ccacaaattt
1801 gataagcaaa aactgtatta ccatgccaca aaagctatga cgcactttac agatgaatgt
1861 gttaaaaagt atgaggcatt tttgaataag tcagaagaat ggataagaaa gatgcttcat
1921 cttaggaaac agttattatc gctgactaat cagtgttttg atattgaaga agaagtatca
1981 aaatatcaag aatatactaa tgagttacaa gaaactctgc ctcagaaaat gtttacagct
2041 tccagtggaa tcaaacatac catgacccca atttatccaa gttctaacac attagtagaa
2101 atgactcttg gtatgaagaa attaaaggaa gagatggaag gggtggttaa agaacttgct
2161 gaaaataacc acattttaga aaggtttggc tctttaacca tggatggtgg ccttcgcaac
2221 gttgactgtc tttag
```

Reviewed and approved by site quality representative.

Unless otherwise stated in our catalogue or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

© 2014 **Eurofins Pharma Discovery Services UK Limited** is an independent member of Eurofins Discovery Services