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Certificate of Analysis

TLK1, active
(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-938, 14-938-K, 14-938M

Parent Lot # D13JP005N

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 TLK1 full length, expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography.
Purity 89% by SDS-PAGE and Coomassie blue staining. MW = 91kDa.

Specific Activity (Parent lot# D13JP005N): 293U/mg, where one unit of TLK1, active activity is defined as 1nmol phosphate incorporated into casein per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.823mg/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.

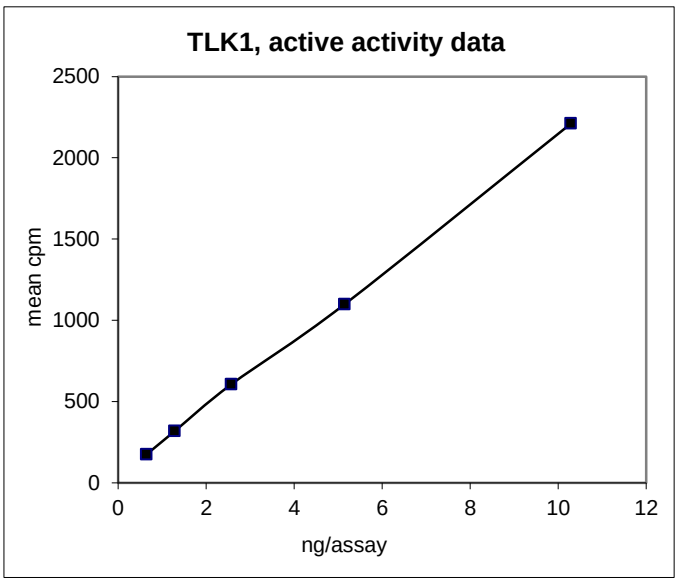
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

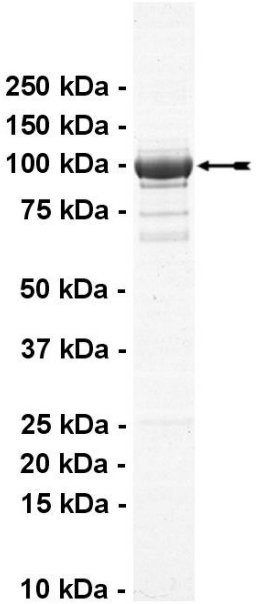
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 0.6–10ng of this lot of enzyme phosphorylated casein 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 TLK1 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 TLK1, active.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **Casein**: Use at a final assay concentration of 2mg/ml. Prepare a 20mg/ml stock and add 2.5µl of stock per assay point.
3. **TLK1, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.6–10ng per assay point.
4. **[γ-^{33}P]ATP:** 2.5 x MgAc/[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 casein.
3. Add **2.5µl (0.6–10ng) TLK1, 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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TLK1 Sequence Information

Protein human TLK1

Tags *N*-terminal 6His

Native sequence M31 of the recombinant protein is equivalent to M1 of human TLK1

Accession number GenBank NM_012290

Recombinant TLK1 amino acid sequence:

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  1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF MSVQSSSGSL EGPPSWSQLS TSPTPGSAAA
 61 ARSLLNHTPP SGRPREGAMD ELHSLDPRRQ ELLEARFTGV ASGSTGSTGS CSVGAKASTN
121 NESSNHSFGS LGSLSDKESE TPEKKQSESS RGRKRKAENQ NESSQGKSIG GRGHKISDYF
181 EYQGGNGSSP VRGIPPAIRS PQNSHSHSTP SSSVRPNSPS PTALAFGDHP IVQPKQLSFK
241 IIQTDLTMLK LAALESNKIQ DLEKKEGRID DLLRANCDLR RQIDEQQKLL EKYKERLNKC
301 ISMSKKLLIE KSTQEKLSSR EKSMQDRLRL GHFTTVRHGA SFTEQWTDGF AFQNLVKQQE
361 WVNQQREDIE RQRKLLAKRK PPTANNSQAP STNSEPKQRK NKAVNGAEND PFVRPNLPQL
421 LTLAEYHEQE EIFKLRLGHL KKEEAEIQAE LERLERVRNL HIRELKRINN EDNSQFKDHP
481 TLNERYLLLH LLGRGGFSEV YKAFDLYEQR YAAVKIHQLN KSWRDEKKEN YHKHACREYR
541 IHKELDHPRI VKLYDYFSLD TDTFCTVLEY CEGNDLDFYL KQHKLMSEKE ARSIVMQIVN
601 ALRYLNEIKP PIIHYDLKPG NILLVDGTAC GEIKITDFGL SKIMDDDSYG VDGMDLTSQG
661 AGTYWYLPPE CFVVGKEPPK ISNKVDVWSV GVIFFQCLYG RKPFGHNQSQ QDILQENTIL
721 KATEVQFPVK PVVSSEAKAF IRRCLAYRKE DRFDVHQLAN DPYLLPHMRR SNSSGNLHMA
781 GLTASPTPPS SSIITY
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Recombinant TLK1 nucleotide sequence:

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   1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
  61 tattttcagg gcgccatgga tccggaattc atgagtgtcc aaagtagcag tggaagtttg
 121 gaggggccgc catcttggtc ccagctctcc acgtctccaa ccccgggctc ggcggcggcg
 181 gccaggtccc tgctgaatca cacgccgcca tccgggaggc ccagggaagg tgcaatggat
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 361 aacgaaagct ctaatcacag ttttggaagc ttgggatctt taagtgacaa agaatcagag
 421 acaccggaga agaaacaatc ggaatcatcc aggggaagaa agagaaaagc agaaaaccag
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 541 gaataccagg gtggaaatgg ctcaagtcca gtaagaggca tacctcctgc aatccgttct
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 661 cctactgcat tagcatttgg ggaccaccct attgtacaac caaagcaatt atcctttaaa
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 901 atatcaatga gcaagaaact tcttattgaa aagagtacac aagaaaagct gtcaagcaga
 961 gagaagagta tgcaagatcg attacgcctt gggcacttta caacagttag acatggcgct
1021 tcatttactg aacaatggac agatggtttt gcatttcaga atcttgtgaa gcaacaagaa
1081 tgggtgaatc agcaaaggga agatattgaa aggcaaagga aacttctagc caaacgcaaa
1141 cctcccacag ctaataattc tcaggcaccc tctaccaatt ctgaaccaaa acaaaggaaa
1201 aacaaagcag tcaatggagc agagaatgat ccctttgtta gaccaaattt accacaactg
1261 ttgactttgg cagaatatca tgaacaggaa gaaattttca aacttagact aggacatctc
1321 aaaaaggaag aggcagaaat ccaggcagaa cttgaacgtt tggaaagagt cagaaatctt
1381 cacatacgtg agctgaaaag aataaacaat gaagataatt cacagttcaa agatcaccca
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1441 acattaaatg aaagatattt attacttcat ctgcttggta gaggtggctt tagtgaagtg
1501 tataaggctt ttgaccttta tgaacaaaga tatgctgctg tgaagataca tcagcttaat
1561 aaaagctgga gagatgagaa gaaagaaaac taccacaaac atgcctgcag agagtataga
1621 atacacaaag aactggatca ccccagaata gttaaactct atgattattt ctccttggat
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1741 aagcaacaca agttaatgtc agagaaagaa gctcggtcta ttgtaatgca gattgtaaat
1801 gcactaagat atctcaatga gatcaaaccc cctattatac attatgatct taagccagga
1861 aacatcctac tggtagatgg aacagcatgt ggtgaaatca aaatcactga ttttggtctg
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2101 agaaagccat ttggtcacaa tcaatctcaa caagacattc ttcaagaaaa tacaatatta
2161 aaagccacag aagtccagtt ccctgtaaaa ccggttgtaa gcagtgaagc caaggcattt
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Reviewed and approved by site quality representative.