

Certificate of Analysis

LTK, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-958, 14-958-K, 14-958M

Parent Lot # D15CP003N

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 GST-tagged, recombinant, human LTK amino acids 450–end expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose followed by gel filtration.

Purity 94% by SDS-PAGE and Coomassie blue staining. MW = 74kDa.

Specific Activity (Parent lot# D15CP003N): 1779U/mg, where one unit of LTK, active activity is defined as 1nmol phosphate incorporated into 250 μ M (GEEPLYWSFPAKKK) per minute at 30°C with a final ATP concentration of 100 μ M.

Formulation: 0.53mg/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% β -mercaptoethanol. Frozen solution.

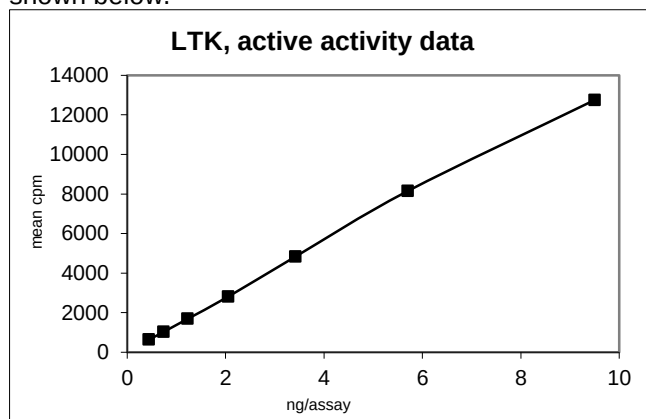
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

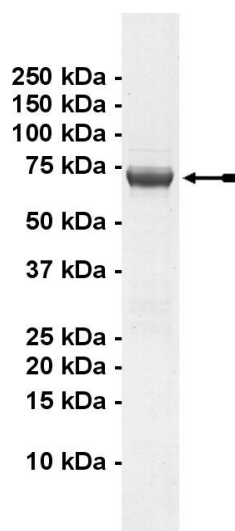
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 0.4–9.5ng of this lot of enzyme phosphorylated 250 μ M (GEEPLYWSFPAKKK) 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 LTK with the translated sequence listed on page three



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3 μ g of LTK, active

Certificate of Analysis

Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 100mM Tris/HCl pH8.5, 1mM EDTA.
2. **(GEEPLYWSFPAKKK):** Use at a final assay concentration of 250 μ M. Prepare a 2.5mM stock and add 2.5 μ l of stock per assay point.
3. **Tween 20:** Use at a final assay concentration of 0.05%. Prepare a 10% stock and add 0.125 μ l per assay point.
4. **BSA:** Use at a final assay concentration of 0.5%. Prepare a 10% stock and add 1.25 μ l per assay point.
5. **LTK, active:** Dilute with 20mM Tris/HCl pH8.5, 0.2mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% β -mercaptoethanol, 1mg/ml BSA. Use 0.4–9.5ng per assay point.
6. **[γ -³³P]ATP:** 2.5 x MgAc/[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 3.625 μ l of dH₂O.
3. Add 0.125 μ l of 10% Tween 20.
4. Add 1.25 μ l of 10% Bovine Serum Albumin (BSA).
5. Add 2.5 μ l of **(GEEPLYWSFPAKKK)**.
6. Add 2.5 μ l (**0.4–5.9ng**) **LTK, active**.
7. Add 10 μ l of diluted [γ -³³P]ATP mixture.
8. Incubate for 10 minutes at 30°C.
9. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
10. Transfer a 10 μ l aliquot onto the appropriate area of a P30 Filtermat.
11. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
12. Wash the filtermat once for 2 minutes with methanol.
13. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
14. 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.

Certificate of Analysis

LTK Sequence Information

<u>Protein</u>	Human LTK
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	K237 of the recombinant protein is equivalent to K450 of human LTK.
<u>Accession number</u>	GenBank NM_002344.3

Recombinant LTK amino acid sequence:

1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID
61	GDVKLTQSM	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV
121	DFLSKLPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK
181	KRIEAIPOID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	LEVLFQGPEF	KGLRRHKQKK
241	WQGLQEMRLP	SPELELSKLR	TSAIRTAPNP	YYCQVGLGPA	QSWPLPPGVT	EVSPANVTLL
301	RALGHGAFGE	VYEGLVIGLP	GDSSPLQVAI	KTLPELCSPQ	DELDFLMEAL	IISKFRHQNI
361	VRCVGLSLRA	TPRLILLELM	SGGDMKSFLR	HSRPHLGQPS	PLVMRDLLQL	AQDIAQGCHY
421	LEENHFIHRD	IAARNCLLSC	AGPSRVAKIG	DFGMARDIYR	ASYYYRGDRA	LLPVKWWPPE
481	AFLEGIFTSK	TDSWSFGVLL	WEIFSLGYMP	YPGRTNQEV	DFVVGGGRMD	PPRGCPGPVY
541	RIMTQCWQHE	PELRPSFASI	LERLQYCTQD	PDVLNSLLPM	ELGPTPEEEG	TSGLGNRSLE
601	CLRPPQPQEL	SPEKLKSWGG	SPLGPWLSSG	LKPLKSRGLQ	PQNLWNPTYR	S

Recombinant LTK nucleotide sequence:

1	atgtccccta	tactaggtta	ttggaaaatt	aagggccttg	tgcaaccac	tcgacttctt
61	ttggaatata	ttgaagaaaa	atatgaagag	catttgatg	agcgcatga	aggtgataaa
121	tggcgaaaca	aaaagtgtga	attgggtttg	gagtttccca	atcttcctta	ttatattgat
181	ggtgatgtta	aattaacaca	gtctatggcc	atcatacgtt	atatagctga	caagcacaac
241	atgttggttg	gttgtccaaa	agagcgtgca	gagatttcaa	tgcttgaagg	agcggttttg
301	gatattagat	acggtgtttc	gagaattgca	tatagtaaag	actttgaaac	tctcaaagtt
361	gatttttctta	gcaagctacc	tgaaatgctg	aaaatgttcg	aagatcgttt	atgtcataaa
421	acataatttaa	atggtgatca	tgtaacccat	cctgacttca	tggtgtatga	cgctcttgat
481	gttggttttat	acatggaccc	aatgtgcctg	gatgcgttcc	caaaattagt	ttgttttaaa
541	aaacgtattg	aagctatccc	acaaaattgat	aagtacttga	aatccagcaa	gtatatagca
601	tggccttttg	agggctggca	agccacgttt	ggtggtggcg	accatcctcc	aaaatcggat
661	ctggaagtgc	tggtccaggg	gcccgaattc	aaaggcctac	gtcgacataa	gcagaagaag
721	tgcaggggcc	tcaggagagat	gaggctgcg	agccctgagc	ttgagctgag	caagcttcga
781	acctctgcca	tcaggacagc	ccccaatccc	tattattgcc	aggtggggct	tggcccggcc
841	cagtcctggc	ctctgccacc	aggtgtcacc	gaggtttccc	cagccaatgt	tactctgctc
901	agagccctgg	gccatggtgc	ctttggggag	gtgtatgagg	gactggtaat	tggccttcct
961	ggggactcca	gtccccctgca	ggtagctatc	aagaccctgc	cagaactctg	ctcgccctcag
1021	gatgagctgg	atttcctcat	ggaggccctc	atcatcagca	agtttcgcca	tcagaacatt
1081	gtgcggtgtg	tggggctcag	cctcagggcc	accctcgc	tcattctgct	ggaactgatg
1141	tctggagggg	acatgaagag	tttcctgagg	cacagtcggc	cacacctggg	ccagccatca
1201	cctctggtca	tgcgggacct	gctgcaactg	gccagggaca	tagcccaggg	ctgccactac
1261	ctggaggaaa	atcacttcat	ccacagggat	attgccgccc	ggaactgcct	gctgagctgc
1321	gctggaccca	gccgagtggc	caagattggg	gactttggga	tggcacgaga	tatctaccgg
1381	gccagttatt	accgcagggg	ggaccggggc	ttgctcccag	tcaagtggat	gccccagag
1441	gccttcctgg	agggcatctt	cacatccaag	acagattcct	ggtcttttgg	ggtgctgctc
1501	tgggagatct	tctcactggg	ctacatgccc	tatcctgggc	gcaccaacca	ggaggtgctg
1561	gacttcgtcg	ttggaggagg	ccggatggac	cctcctaggg	gctgcccagg	gcctgtgtac
1621	cgcatcatga	cccagtgttg	gcagcacgag	cctgagctcc	gccctagctt	tgccagcatc
1681	ttggagcgct	tgcagtactg	cactcaggac	ccggatgtgc	tgaattcact	ctcgccaatg
1741	gagctggggc	ccaccccaga	ggaggaaggg	acttctgggc	tggggaacag	atctttggag
1801	tgccctaagac	ccccacagcc	ccagggaactg	agtccagaga	agttgaaaaag	ctggggaggt

Certificate of Analysis

```
1861 agccctcttg gccctggct gtcctctggc ctcaagcccc tcaaatccag gggcctccaa  
1921 cctcagaacc ttggaatcc cacttatcgc tcctga
```

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