

## Certificate of Analysis

### MLK1, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-690, 14-690-K, 14-690M

Parent Lot # WAB0390

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 MLK1, amino acids 134–414, expressed by baculovirus in Sf21 insect cells. Purified using Ni<sup>2+</sup>/NTA agarose. Purity 77% by SDS-PAGE and Coomassie blue staining. MW = 35.3kDa.

**Specific Activity (Parent lot# WAB0390):** 293U/mg, where one unit of MLK1, active activity is defined as 1nmol phosphate incorporated into 2mg/ml casein per minute at 30°C with a final ATP concentration of 100µM.

**Formulation:** 0.59mg/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.

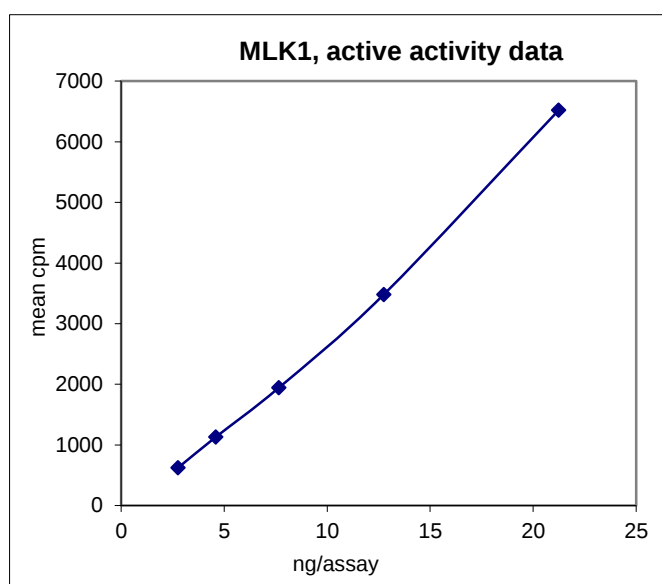
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**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.

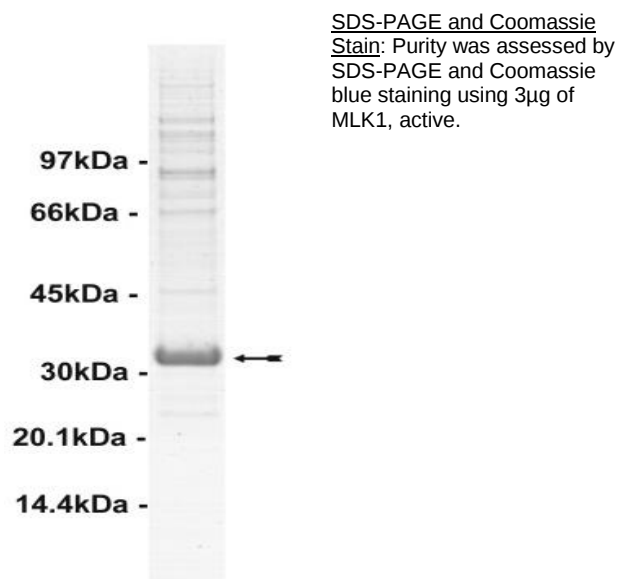
**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### Quality Control Testing

**Kinase Assay:** 2.8–21.2ng of this lot of enzyme phosphorylated 2mg/ml 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 MLK1 with the translated native sequence listed on page three.



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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 concentration of 2mg/ml. Make a 20mg/ml stock. Use 2.5µl per assay point.
3. **MLK1, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 2.8–21.2ng per assay point.
4. **[ $\gamma$ -<sup>33</sup>P]ATP:** 2.5 x magnesium acetate/[ $\gamma$ -<sup>33</sup>P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [ $\gamma$ -<sup>33</sup>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 (2.8–21.2ng) MLK1, active**.
4. Add 5µl of dH<sub>2</sub>O.
5. Add 10µl of diluted [ $\gamma$ -<sup>33</sup>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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### MLK1 Sequence Information

|                                |                                                                       |
|--------------------------------|-----------------------------------------------------------------------|
| <b><u>Protein</u></b>          | Human MLK1                                                            |
| <b><u>Tags</u></b>             | N-terminal 6His                                                       |
| <b><u>Native sequence</u></b>  | I31 of recombinant protein is equivalent to I134 of native human MLK1 |
| <b><u>Accession number</u></b> | GenBank NM_033141                                                     |

#### ***Recombinant MLK1 amino acid sequence:***

```

1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF IQLLEIDFAE LTLEEIIGIG GFGKVYRAFW
61 IGDEVAVKAA RHPDDEISQ TIENVRQEA LFAMLKHPNI IALRGVCLKE PNLCLVMEFA
121 RGGPLNRVLS GKRIPPDILV NWAVQIARGM NYLHDEAIVP IIHRDLKSSN ILILQKVENG
181 DLSNKILKIT DFGLAREWHR TTKMSAAGTY AWMapevira SMFSKGSVDW SYGVLLWELL
241 TGEVPFRGID GLAVAYGVAM NKLALPIST CPEPFAKLME DCWNPDPHSR PSFTNILDQL
301 TTIEESGFFE M

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#### ***Recombinant MLK1 nucleotide sequence:***

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tatttttcagg gcgccatgga tccggaattc attcagttgt tagaaattga ttttgcggag
121 ctcaccttgg aagagattat tggcatcggg ggctttggga aggtctatcg tgctttctgg
181 ataggggatg aggttgctgt gaaagcagct cgccacgacc ctgatgagga catcagccag
241 accatagaga atgttcgcca agaggccaag ctcttcgcca tgctgaagca ccccaacatc
301 attgccctaa gaggggatg tctgaaggag cccaacctct gcttggtcat ggagtttgct
361 cgtggaggac ctttgaatag agtgttatct gggaaaagga ttccccaga catcctgggtg
421 aattgggctg tgcagattgc cagagggatg aactacttac atgatgaggc aattgttccc
481 atcatccacc gcgaccttaa gtccagcaac atattgatcc tccagaagggt ggagaatgga
541 gacctgagca acaagattct gaagatcact gattttggcc tggctcggga atggcaccga
601 accaccaaga tgagtgcggc agggacgtat gcttggtatg caccggaagt catccgggcc
661 tccatgtttt ccaaaggcag tgatgtgtgg agctatgggg tgctactttg ggagttgctg
721 actggtgagg tgccctttcg aggcattgat ggcttagcag tcgcttatgg agtggccatg
781 aacaaactcg cccttcctat tccttctacg tgccagaac cttttgcca actcatggaa
841 gactgctgga atcctgatcc ccactcacga ccatctttca cgaatatacct ggaccagcta
901 accaccatag aggagtctgg tttctttgaa atgtaa

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