

Certificate of Analysis

LOK, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-686, 14-686-K, 14-686M

Parent Lot # 2210533

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 LOK amino acids 1–348, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 67.7% by SDS-PAGE and Coomassie blue staining. MW = 43.2kDa.

Specific Activity (Parent lot# 2210533): 885U/mg, where one unit of LOK, active activity is defined as 1nmol phosphate incorporated into 250μM (KKSREGDYMTMQIG) per minute at 30°C with a final ATP concentration of 100μM.

Formulation: 0.847mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 20mM β-glycerophosphate, 0.25mM sodium orthovanadate, 10mM NaF 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

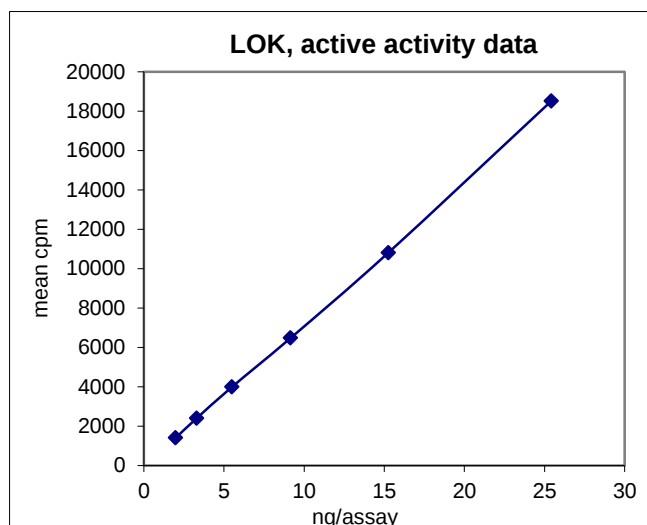
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

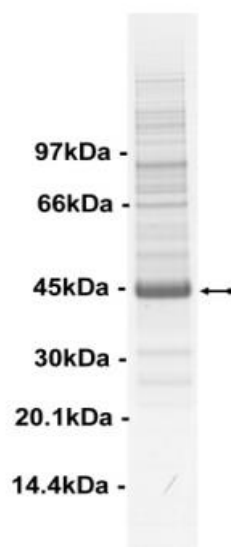
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 2–25.4ng of this lot of enzyme phosphorylated 250μM (KKSREGDYMTMQIG) 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 LOK 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 LOK, active.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS-NaOH pH7.0, 1mM EDTA.
2. **(KKS RGDYMTMQIG):** Use at a final assay concentration of 250µM. Prepare a 2.5mM stock. Add 2.5µl of stock per assay point.
3. **LOK, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 2–25.4ng per assay point.
4. **[γ-³³P]ATP:** 2.5 x magnesium acetate/[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 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **(KKS RGDYMTMQIG)**.
3. Add **2.5µl (2–25.4ng) LOK, active**.
4. Add 5µl of dH₂O.
5. Add 10µl of diluted [γ-³³P]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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LOK Sequence Information

<u>Protein</u>	Human LOK
<u>Tags</u>	N-Terminal 6His
<u>Native sequence</u>	M31 of recombinant protein is equivalent to M1 of human LOK
<u>Accession number</u>	GenBank NM_005990

Recombinant LOK amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF MAFANFRRL RLSTFEKRKS REYEHVRRDL
61 DPNEVWEIVG ELGDGAFGKV YKAKNKETGA LVAKVVIETK SEEELEDYIV EIEILATCDH
121 PYIVKLLGAY YHDGKLWIMI EFCPGGAVDA IMLELDRLGT EPQIQEVCRQ MLEALNFLHS
181 KRIIHRDLKA GNVLMTLEGD IRLADFGVSA KNLKTLQKRD SFIGTPYWMA PEVVMCETMK
241 DTPYDYKADI WSLGITLIEM AQIEPPHEL NPMRVLLKIA KSDPPTLLTP SKWSVEFRDF
301 LKIALDKNPE TRPSAAQLLE HPFVSSITSN KALRELVAEA KAEVMEGIED GRDEGEEDDA
361 VDAASTLENH TQNSSEVS

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Recombinant LOK nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatgga tccggaattc atggcttttg ccaatttcg cgcacatcctg
121 cgctgtctta ccttcgagaa gagaaagtc cgcgaatat agcacgtccg ccgcgacctg
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421 gagttctgtc cagggggagc cgtggagccc atcatgctgg agctggacag aggcctcacg
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