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Certificate of Analysis

ULK1, active
(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-959, 14-959-K, 14-959M

Parent Lot # D15BP002N

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 ULK1 amino acids 1-314 expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography followed by gel filtration.
Purity 83% by SDS-PAGE and Coomassie blue staining. MW = 39kDa.

Specific Activity (Parent lot# D15BP002N): 2858U/mg, where one unit of ULK1 activity is defined as 1nmol phosphate incorporated into 2mg/ml casein per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.45mg/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.

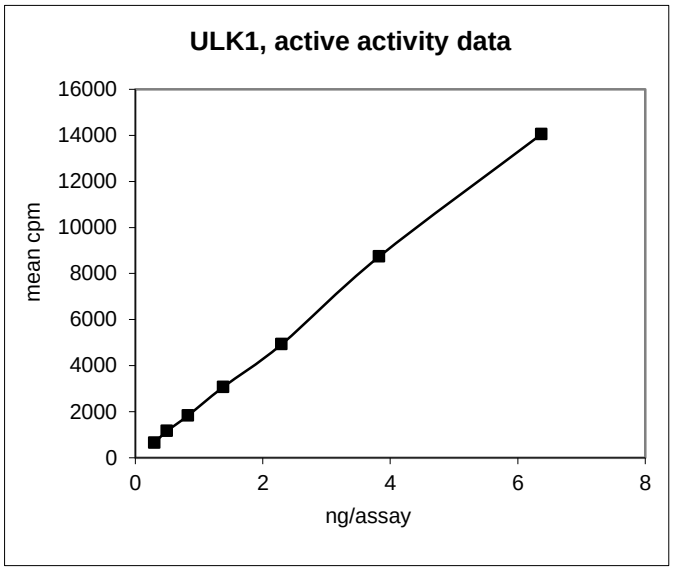
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

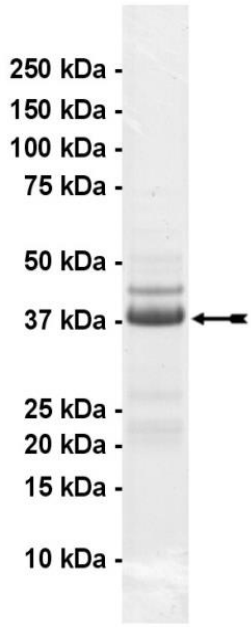
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 0.3–6.4ng 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 ULK1 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 ULK1, 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. **ULK1, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.3–6.4ng 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 2mg/ml casein.
3. Add **2.5µl (0.3–6.4ng) ULK1, 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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ULK1, active Sequence Information

Protein human ULK1

Tags *N*-terminal 6His

Native sequence M31 of the recombinant protein is equivalent to M1 of human ULK1.

Accession number GenBank NM_003565.1

Recombinant ULK1 amino acid sequence:

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   1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF MEPGRGGTET VGKFEFSRKD LIGHGAFAVV
  61 FKGRHREKHD LEVAVKCINK KNLAKSQTLL GKEIKILKEL KHENIVALYD FQEMANSVYL
 121 VMEYCNGGDL ADYLHAMRTL SEDTIRLFLQ QIAGAMRLLH SKGIIHRDLK PQNILLSNPA
 181 GRRANPNSIR VKIADFGFAR YLQSNMMAAT LCGSPMYMAP EVIMSQHYDG KADLWSIGTI
 241 VYQCLTGKAP FQASSPQDLR LFYEKNKTLV PTIPRETSAP LRQLLLALLQ RNHKDRMDFD
 301 EFFHHPFLDA SPSVRKSPPV PVPSYPSSGS GSSSSSSSTS HLAS
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Recombinant ULK1 nucleotide sequence:

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   1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
  61 tattttcagg gcgccatgga tccggaattc atggagcccg gccgcggcgg cacagagacc
 121 gtgggcaagt tcgagttctc ccgcaaggac ctgatcggcc acggcgcctt cgcggtggtc
 181 ttcaagggcc gccaccgcga gaagcacgat ttggaggtcg ccgtcaagtg cattaacaag
 241 aagaacctcg ccaagtctca gacgctgctg gggaaggaaa tcaaaatcct gaaggaactg
 301 aaacatgaaa acatcgtggc cctgtacgac ttccaggaaa tggctaattc tgtctacctg
 361 gttatggagt actgcaacgg tggggacctg gccgactacc tgcacgccat gcgcacgctg
 421 agcgaggaca ccatcaggct cttcctgcag cagatcgcgg gcgccatgcg gcttctgcac
 481 agcaaaggca tcatccaccg cgacctgaaa ccgcagaaca tcctgctgtc caaccccgcc
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 721 gtctaccagt gcctgacggg gaaggcgccc ttccaggcca gcagccccca ggacctgcgc
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 961 cctgtgccct cgtacccaag ctcggggtcc ggcagcagct ccagcagcag ctccacctcc
1021 cacctggcct cctaa
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Reviewed and approved by site quality representative.