

Certificate of Analysis

ULK2, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-772, 14-772-K, 14-772M

Parent Lot # WAE0379

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 and C-terminal 6His-tagged, recombinant, human, ULK2, amino acids 1–306. Expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose. Purity 94% by SDS-PAGE and Coomassie staining. MW = 62kDa.

Specific Activity (Parent lot# WAE0379): 439U/mg, where one unit of ULK2, 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: 0.23mg/ml of enzyme in 50mM Tris/HCl pH7.2, 300mM NaCl, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1mM EGTA, 0.1% 2-mercaptoethanol, 0.03% Brij-35. Frozen solution.

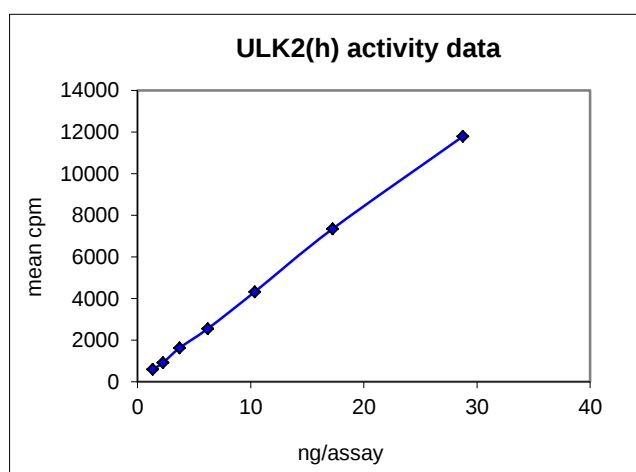
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

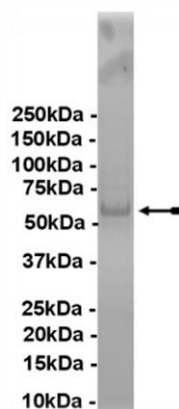
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 1.34–28.75ng 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 product identity as ULK2 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 ULK2, 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. Make up a 20mg/ml stock. Add 2.5µl of stock per assay point.
3. **ULK2, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1.34–28.75ng 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 **casein**.
3. Add **2.5µl (1.34–28.75ng) ULK2, 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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ULK2 Sequence Information

Protein	Human ULK2
Tags	N-terminal GST and C-terminal 6His
Native sequence	M231 of recombinant sequence is equivalent to M1 of native human ULK2
Accession number	GenBank NM_014683

Recombinant ULK2 amino acid sequence:

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1  MSPILGYWKI KGLVQPTRL L LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPPYID
61  GDVKLTQSM A IIRYIADKH N MLGGCPKERA EISMLEGAVL DIRYGVSR I YSKDFETLKV
121 DFLSKLPEML KMFEDRLCH K TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAI PQID KYLKSSKYI A WPLQGWQATF GGGDHPPKSD LEVLFQGPEF MEVVGDFEYS
241 KRDLVGHGAF AVVFRGRHR Q KTDWEVAIKS INKKNLSKSQ ILLGKEIKIL KELQHENIVA
301 LYDVQELPNS VFLVMEYCNG GDLADYLQAK GTLSEDTIRV FLHQIAAAMR ILHSGKIIHR
361 DLKPQNILLS YANRRKSSVS GIRIKIADFG FARYLHSNMM AATLCGSPMY MAPEVIMSQH
421 YDAKADLWSI GTVIYQCLVG KPPFQANSPQ DLRMFYEKNR SLMPSIPRET SPYLANLLLG
481 LLQRNQKDRM DFEAFFSHPF LEQGPVKKSC PVPVPMYSGS VSGSSCGSSP SCR FASHHHH
541 HH

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Recombinant ULK2 nucleotide sequence:

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1  atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaaccac tcgacttctt
61  ttggaatatc ttgaagaaaa atatgaagag catttgatg agcgcatga aggtgataaa
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241 atgttgggtg gttgtccaaa agagcgtgca gagattcaa tgcttgaagg agcggttttg
301 gatattagat acggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
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1621 caccattaa

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