

## Certificate of Analysis

### ULK3, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-755, 14-755-K, 14-755M

Parent Lot # D8KN003U

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 full-length ULK3. Expressed by baculovirus in Sf21 insect cells. Purified using Ni<sup>2+</sup>/NTA agarose. Purity 91.7% by SDS-PAGE and Coomassie staining. MW = 62kDa.

**Specific Activity (Parent lot# D8KN003U):** 302U/mg, where one unit of ULK3, 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:** 1.368mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1mM EGTA, 0.1% 2-mercaptoethanol, 0.03% Brij-35. Frozen solution.

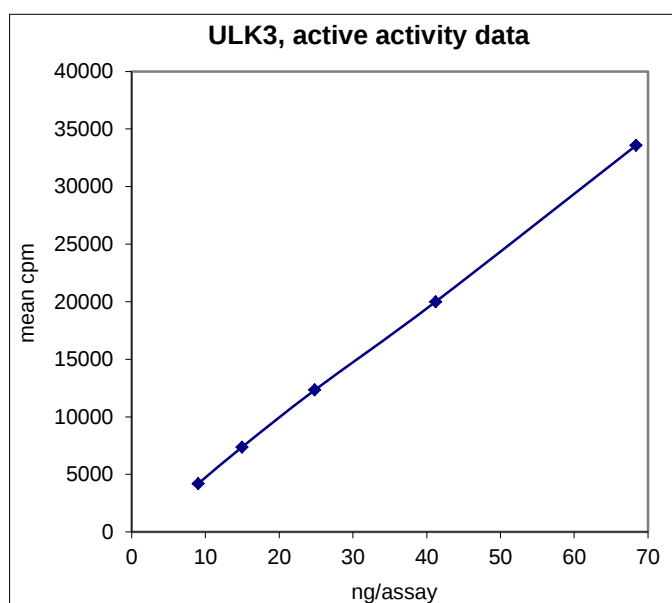
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**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.

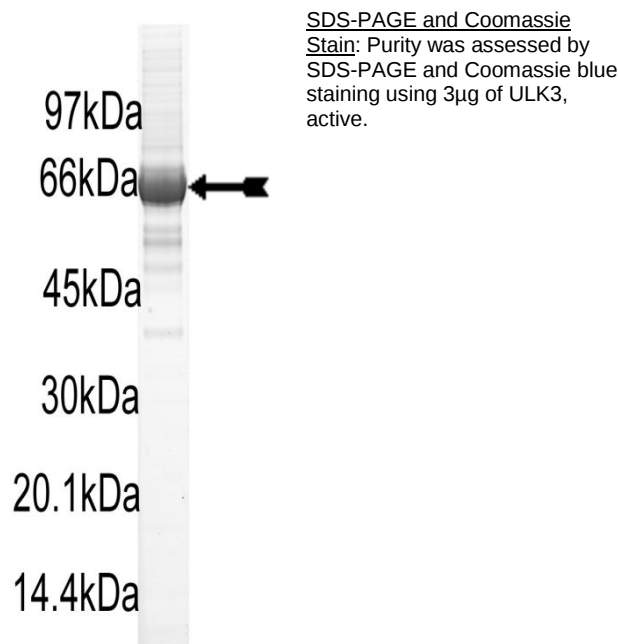
**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### Quality Control Testing

**Kinase Assay:** 9–68ng 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 ULK3 with the translated 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 assay concentration of 2mg/ml. Make up a 20mg/ml stock. Add 2.5µl of stock per assay point.
3. **ULK3, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 9–68ng 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 (9–68ng) ULK3, 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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## ULK3 Sequence Information

|                         |                                                                              |
|-------------------------|------------------------------------------------------------------------------|
| <b>Protein</b>          | Human ULK3                                                                   |
| <b>Tags</b>             | N-terminal 6His                                                              |
| <b>Native sequence</b>  | M34 of recombinant sequence is equivalent to M1 of native human ULK3         |
| <b>Accession number</b> | Gen Bank XM_044630 (DNA has been codon optimized for insect cell expression) |

### Recombinant ULK3 amino acid sequence:

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1 MSYYHHHHH DYDIPTTENL YFQGAMDPEF GRRMRAAFPA GGAGGSVEPP SARPAPQPAG
61 TAARSEEAPA RAQAAGMAGP GWGPPRLDGF ILTERLGSGT YATVYKAYAK KDTREVVAIK
121 CVAKKS LNKA SVENLLTEIE ILKGIRHPHI VQLKDFQWDS DNIYLIMEFC AGGDL SRFIH
181 TRRILPEKVA RVFMQQLASA LQFLHERNIS HLDLKPQNIL LSSLEKPHLK LADFGFAQHM
241 SPWDEKHVLR GSPLYMAPEM VCQRQYDARV DLWSMGVILY EALFGQPPFA SRSFSELEEK
301 IRSNRVIELP LRPLLSRDCR DLLQRLLED RPSRRISFQDF FAHPWVDLEH MPSGESLGRA
361 TALVVQAVKK DQEGDSAAAL SLYCKALDFF VPALHYEVDA QRKEAIKAKV GQYVSRAEEL
421 KAIVSSSNQA LLRQGTSARD LLREMARKDP RLLAALEVAS AAMAKEEAAG GEQDALDLYQ
481 HSLGELL LLL AAEPGRRRE LLHTEVQNLM ARAEYLKEQV KMRESRWEAD TLDKEGLSES
541 VRSSCTLQ

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### Recombinant ULK3 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatgga tccagaattc ggtcgacgaa tgagagctgc atttccagca
121 ggaggagccg gaggatctgt tgaacccct tctgcaagac ccgccccaca acctgccgga
181 accgctgctc gctcagaaga agcaccagct agggcccagg ctgctggcat ggccggacca
241 ggaatggggac ctcccaggct ggacggtttc atcctgactg agaggctggg atctggtacc
301 tacgccaccg tttaaaaagc atacgccaag aaggacacta gggaggtcgt tgctatcaaa
361 tgcgtggcca aaaagtcatt gaacaaggcc tccgtcgaat atctctcac tgaaattgag
421 atcctgaagg gcatcaggca tccacacatc gttcagctca aagacttcca atgggactct
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601 ttgcaatttc tccatgagag aaacatcagc cacctggatt tgaagcccca gaacattttg
661 ctgtctagcc tggagaagcc tcacctcaaa ctggccgatt ttggattcgc tcagcacatg
721 tctccttggg acgagaaaca tgtgttgagg ggaagccctc tgtacatggc tcctgaaatg
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1021 ttcgctcatt cttgggtgga tctggaacac atgccatctg gagagtcctt gggtagggct
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1141 tctctgtatt gaaaagcttt ggactttttc gtgcccgtc tccactacga agtggagcc
1201 cagcgcaaa aggcaatcaa ggctaagggt ggtcagtagc tctcaagagc tgagggaattg
1261 aaagctatcg tttctcaag caaccaagct ctgctcagac agggaacctc tgcccgcgac
1321 ctctgagggt aaatggctag ggacaagcca cgcctcctgg ccgcccctga agtcgctagc
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1441 cattccctgg gagaactcct gctgctgctg gctgccgaac cccaggaag acgcaggag
1501 ctgctgcaca cagaggttca gaacttgatg gcaagggccg agtacttgaa agagcaagtt
1561 aagatgcgcg aatcacgctg ggaagctgac accctggaca aggaaggtct gtccgagagc
1621 gttagggtcaa gctgcacttt gcagtag

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Reviewed and approved by site quality representative.

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