

## Certificate of Analysis

### STK32A, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 16-011, 16-011-K, 16-011M

Parent Lot # D17SP004N

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 STK32A full length, expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography. Purity 85% by SDS-PAGE and Coomassie blue staining. MW = 50kDa.

**Specific Activity (Parent lot# D17SP004N):** 16U/mg, where one unit of STK32A activity is defined as 1nmol phosphate incorporated into 2mg/ml casein per minute at 30°C with a final ATP concentration of 100µM.

**Formulation:** 2.45mg/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.

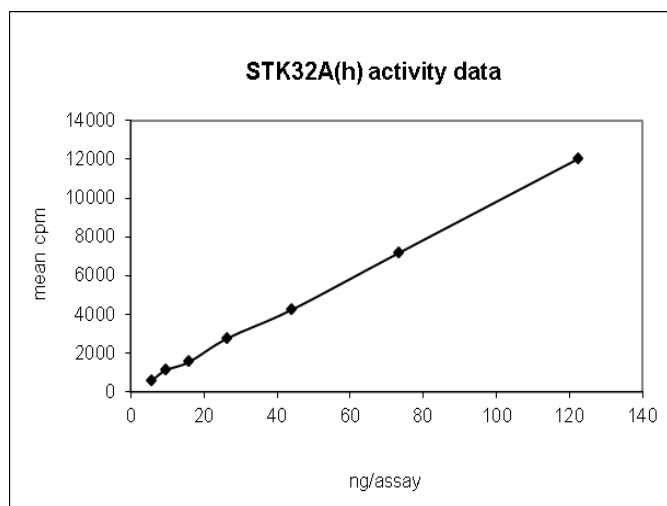
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**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.

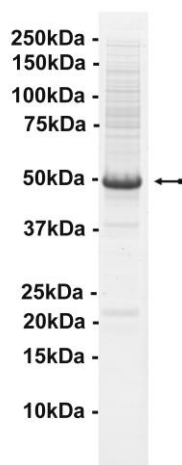
**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### Quality Control Testing

**Kinase Assay:** 5.72–122.50ng 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 STK32A 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 STK32A, active.

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### Kinase Assay Protocol

#### Stock Solutions:

- 1. 5 x Reaction Buffer:** 100mM Tris/HCl pH8.5, 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. STK32A, active:** Dilute with 20mM Tris/HCl pH8.5, 0.2mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 5.72–122.50ng per assay point.
- 4. [ $\gamma$ -<sup>33</sup>P]ATP:** 2.5 x MgAc/[ $\gamma$ -<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 (5.72–122.50ng) STK32A, 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 30 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 dried 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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## STK32A, active Sequence Information

<b><u>Protein</u></b>	Human, STK32A
<b><u>Tags</u></b>	N-terminal 6His
<b><u>Native sequence</u></b>	M31 of the recombinant protein is equivalent to M1 of human STK32A
<b><u>Accession number</u></b>	GenBank NM_001112724.1

### Recombinant STK32A amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF MGANTSRKPP VFDENEDVNF DHFEILRAIG
61 KGSFGKVCIV QKNDTKKMYA MKYMNKQKCV ERNEVRNVFK ELQIMQGLEH PFLVNLWYSF
121 QDEEDMFMVV DLLLGGDLRY HLQQNVHFKE ETVKLFICEL VMALDYLQNO RIIHRDMKPD
181 NILLDEHGHV HITDFNIAAM LPRETQITTM AGTKPYMAPE MFSSRKGAGY SFAVDWWSLG
241 VTAYELLRGR RPYHIRSSTS SKEIVHTFET TVVTYPSAWS QEMVSLKLL LEPNPDQRF
301 QLSDVQNFY MNDINWDAVF QKRLIPGFIP NKGRLNCDPT FELEEMILES KPLHKKKKRL
361 AKKEKDMRKC DSSQTCLLQE HLDSVQKEFI IFNREKVNDR FNKRQPNLAL EQTKDPQGED
421 GQNNNL

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### Recombinant STK32A nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatgga tccggaattc atgggagcca acacttcaag aaaaccacca
121 gtgtttgatg aaaatgaaga tgtcaacttt gaccactttg aaatthttgcg agccattggg
181 aaaggcagtt ttgggaaggt ctgcattgta cagaagaatg ataccaagaa gatgtacgca
241 gaactccaga tcatgcaggg tctggagcac ctttcctgg ttaatttggt gtattccttc
301 caagatgagg aagacatggt catggtggtg gacctcctgc tgggtggaga cctgcgttat
361 cacctgcaac agaacgtcca cttcaaggaa gaaacagtga agctcttcat ctgtgagctg
481 gtcatggccc tggactacct gcagaaccag cgcacattc acagggatat gaagcctgac
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781 agcaaggaaa ttgtacacac gtttgagacg actggtgtaa cttacccttc tgcctggtca
841 caggaaatgg tgtcacttct taaaagcta ctcgaaccta atccagacca acgattttct
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1141 caccttgact ctgtccagaa ggagttcata attttcaaca gagaaaaagt aaacagggac
1201 ttttaacaaa gacaacaaa tctagccttg gaacaaacca aagaccaca aggtgaggt
1261 ggtcagaata acaacttgta a

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