

Certificate of Analysis

STK33, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-671, 14-671-K, 14-671M

Parent Lot # 1688267

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, full length, human STK33, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity = 87.4% by SDS-PAGE and Coomassie blue staining. MW = 62.4kDa.

Specific Activity (Parent lot# 1688267): 32U/mg, where one unit of STK33 activity is defined as 1nmol phosphate incorporated into 0.33mg/ml myelin basic protein per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.621mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamide, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

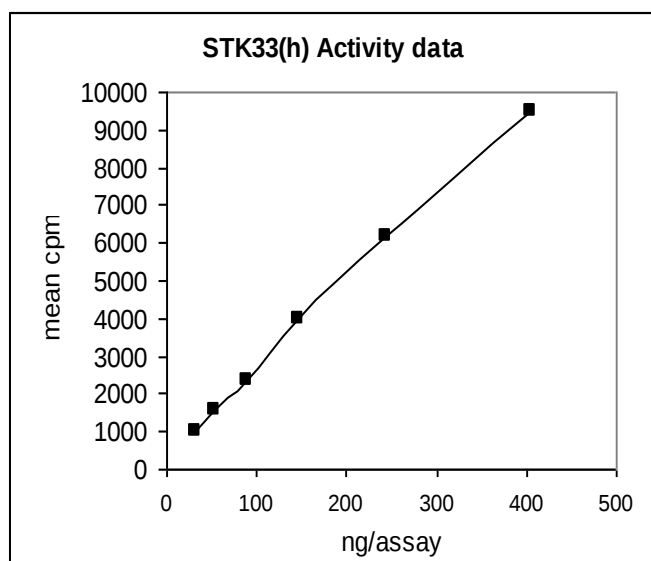
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

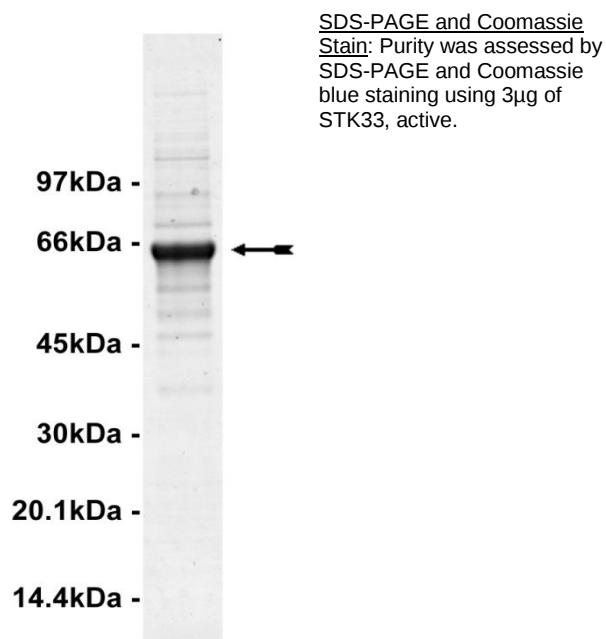
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 32–405ng of this lot of enzyme phosphorylated 0.33mg/ml myelin basic protein 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 STK33 with the translated native 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. Myelin Basic Protein (MBP):** Use at a final assay concentration of 0.33mg/ml. Make up a 3.3mg/ml stock. Use 2.5µl of stock per assay point.
- 3. STK33, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 32–405ng 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 **myelin basic protein (MBP)**.
3. Add **2.5µl (32–405ng) STK33, 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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STK33 Sequence Information

Protein	human STK33
Tags	N-Terminal 6His
Native sequence	M37of recombinant sequence is equivalent to M1 of native human STK33
Accession number	GenBank NM_030906

Recombinant STK33 amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF KGLRRRMADS GLDKKSTKCP DCSSASQKDV
61 LVCSSKTRV PPVLVEMSQ TSSIGSAESL ISLERKKEKN INRDITSRKD LPSRTSNVER
121 KASQQQWGRG NFTEGKVPFI RIENGAAIEE IYTFGRILGK GSFGIVIEAT DKETETKWA
181 KKVNKEKAGS SAVKLLEREV NILKSVKHEH IIHLEQVFET PKKMYLVMEI CEDGELKEIL
241 DRKGHFSENE TRWIIQSLAS AIAYLHNNDI VHRDLKLENI MVKSSLIDDN NEINLNKVT
301 DFGGLAVKKQS RSEAMLQATC GTPIYMAPEV ISAHDSQQC DIWSIGVVMY MLLRGEPPFL
361 ASSEELKFEL IRKGEHFEN AVWNSISDCA KSVLKQLMKV DPAHRITAKE LLDNQWLTGN
421 KLSSVRPTNV LEMMKWKNN PESVEENTTE EKNKPSTEEK LKSYQPWGNV PDANYTSDEE
481 EEKQSTAYEK QFPATSKDNF DMCSSSFTSS KLLPAEIKGE MEKTPVTPSQ GTATKYPKAS
541 GALSRTKKKL

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Recombinant STK33 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatgga tccggaattc aaaggcctac gtcgacgaat ggctgatagt
121 ggcttagata aaaaatccac aaaatgcccc gactgttcac ctgcttctca gaaagatgta
181 ctttgtgtat gttccagcaa aacaagggtt cctccagttt tgggtggtgga aatgtcacag
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1561 atggagaaaa cccctgtgac tccaagccaa ggaacagcaa ccaagtaccc tgctaaatcc
1621 ggcgcctgt ccagaaccaa aaagaaactc taa

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