

## Certificate of Analysis

### STK25, active

#### (Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-929, 14-929-K, 14-929M

Parent Lot # D13CP008N

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-tagged and C-terminal 6His-tagged, recombinant, human STK25 amino acids 1-308, expressed by baculovirus in Sf21 insect cells. Purified using glutathione sepharose. Purity 94% by SDS-PAGE and Coomassie blue staining. MW = 63kDa.

**Formulation:** 0.961mg/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.

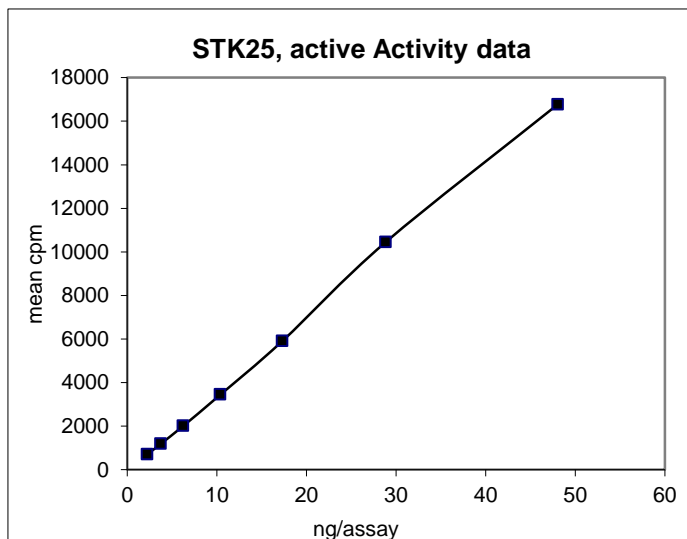
**Specific Activity (Parent lot# D13CP008N):** 382U/mg, where one unit of STK25, active activity is defined as 1nmol phosphate incorporated into 250µM (RLGRDKYKTLRQIRQ) per minute at 30°C with a final ATP concentration of 100µM.

**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.

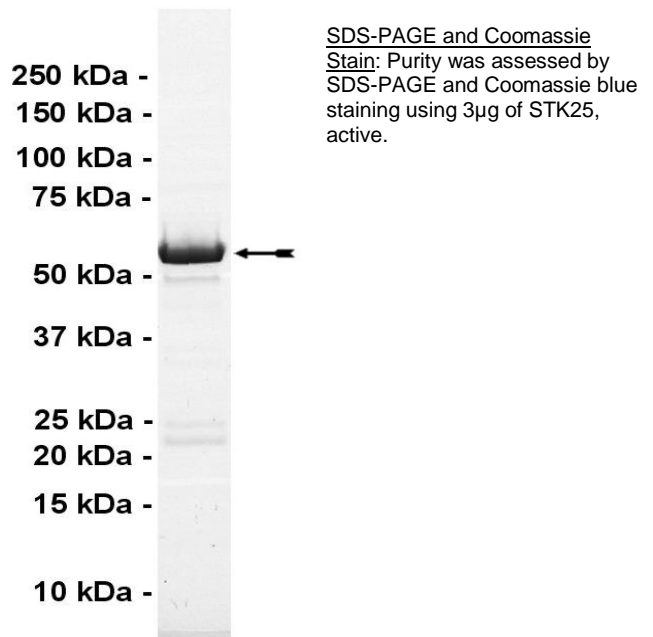
**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### Quality Control Testing

**Kinase Assay:** 2–48ng of this lot of enzyme phosphorylated 250µM (RLGRDKYKTLRQIRQ) 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 STK25 with the translated sequence listed on page three.



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### Kinase Assay Protocol

#### Stock Solutions:

1. **5 x Reaction Buffer:** 100mM Tris/HCl pH8.5, 1mM EDTA.
2. **(RLGRDKYKTLRQIRQ):** Use at a final assay concentration of 250 $\mu$ M. Prepare a 2.5mM stock and add 2.5 $\mu$ l of stock per assay point.
3. **10% BSA:** Use at a final concentration of 1% (w/v). Prepare a 10% solution in water, and add 2.5 $\mu$ l per well
4. **STK25, 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 2–48ng per assay point.
5. **[ $\gamma$ -<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 5 $\mu$ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 $\mu$ l of **(RLGRDKYKTLRQIRQ)**.
3. Add 2.5 $\mu$ l of 10% BSA.
4. Add **2.5 $\mu$ l (2–48ng) STK25, active**.
5. Add 2.5 $\mu$ l of dH<sub>2</sub>O.
6. Add 10 $\mu$ l of diluted [ $\gamma$ -<sup>33</sup>P]ATP mixture.
7. Incubate for 10 minutes at 30°C.
8. Stop the reaction by adding 5 $\mu$ l of 3% phosphoric acid.
9. Transfer a 10 $\mu$ l aliquot onto the appropriate area of a **P30 Filtermat**.
10. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
11. Wash the filtermat once for 2 minutes with methanol.
12. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
13. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1 $\mu$ l of 30% phosphoric acid.

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## STK25 Sequence Information

<b><u>Protein</u></b>	human STK25
<b><u>Tags</u></b>	N-terminal GST and C-terminal 6His.
<b><u>Native sequence</u></b>	M231 of the recombinant protein is equivalent to M1 of human STK25
<b><u>Accession number</u></b>	GenBank NM_006374

### Recombinant STK25 amino acid sequence:

```

1  MSPILGYWKI  KGLVQPTRLL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQSMa  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121 DFLSKLPEML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181 KRIEAIPIQID  KYLKSSKYIA  WPLQGWQATF  GGDHPPKSD  LEVLFQGPEF  MAHLRGFANQ
241 HSRVDPEELF  TKLDRIGKGS  FGEVYKGIN  HTKEVVAIKI  IDLEEADEI  EDIQQEITVL
301 SQCDSPYITR  YFGSYLKSTK  LWIIMEYLG  GSALDLLKPG  PLEETYIATI  LREILKGLDY
361 LHSERKIHRD  IKAANVLLSE  QGDVKLADFG  VAGQLTDTQI  KRNTFVGT  PFWMAPEVIKQS
421 AYDFKADIWS  LGITAIELAK  GEPPNSDLHP  MRVLFILPKN  SPPTLEGQHS  KPFKEFVEAC
481 LNKDPRFRPT  AKELLKHKFI  TRYTKKTSFL  TELIDRYKRW  KSEGHGEES  SEDSDIDGHH
541 HHHH
  
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### Recombinant STK25 nucleotide sequence:

```

1  atgtccccta  tactaggtta  ttggaaaatt  aaggcccttg  tgcaaccac  tcgacttctt
61  ttggaatatc  ttgaagaaaa  atatgaagag  catttgatg  agcgcgatga  aggtgataaa
121  tggcgaaaca  aaaagtttga  attgggtttg  gagtttcca  atcttcctta  ttatattgat
181  ggtgatgtta  aattaacaca  gtctatggcc  atcatacgtt  atatagctga  caagcacaac
241  atgttgggtg  gttgtccaaa  agagcgtgca  gagatttcaa  tgcttgaagg  agcggttttg
301  gatattagat  acggtgtttc  gagaattgca  tatagtaaag  actttgaaac  tctcaaagtt
361  gattttctta  gcaagctacc  tgaatgctg  aaaatgttcg  aagatcgttt  atgtcataaa
421  acatatttaa  atggtgatca  tgtaaccat  cctgacttca  tgttgatga  cgctcttgat
481  gttgttttat  acatggacc  aatgtgcctg  gatgcgttcc  caaaattagt  ttgttttaa
541  aaacgtattg  aagctatccc  acaaattgat  aagtacttga  aatccagcaa  gtatatagca
601  tggcctttgc  agggctggca  agccacgttt  ggtggtggcg  accatcctcc  aaaatcgat
661  ctggaagttc  tgttccaggg  gccgaattc  atggctcacc  tccggggatt  tgccaaccag
721  cactctcgag  tggaccctga  ggagctctc  accaagctcg  accgcattgg  caagggtcgc
781  tttggggagg  tctacaaggg  catcgataac  cacacaaagg  aggtggtggc  catcaagatc
841  atcgacctgg  aggaggccga  ggatgagatc  gaggacatcc  agcaggagat  cactgtcctc
901  agtcagtgcg  acagccccta  catcaccgc  tactttggct  cctacctaaa  gagcaccaag
961  ctatggatca  tcatggagta  cctgggcggc  ggctcagcac  tggacttgct  taaaccaggt
1021  cccctggagg  agacatacat  tgccacgatc  ctgcgggaga  ttctgaaggg  cctggattat
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1141  cagggtgacg  tgaagctggc  ggactttggg  gtagcagggc  agctcacaga  cagcagattt
1201  aagaggaaca  cattcgtggg  cacccttc  tggatggcac  ctgaggtcat  caagcagtgc
1261  gcctacgact  tcaaggctga  catctggctc  ctggggatca  cagccatcga  gctggccaag
1321  ggggagcctc  caaactctga  cctccacccc  atgcgcgtcc  tgttcctgat  tccaagaac
1381  agcccacca  cactggaggg  ccagcacagc  aagcccttca  aggagtctgt  ggaggcctgc
1441  ctcaacaaag  accccgatt  ccggcccag  gccaaaggagc  tcctgaagca  caagttcatc
1501  acacgctaca  ccaagaagac  ctcttctc  acggagctca  tcgaccgcta  taagcgtcgg
  
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## Certificate of Analysis

1561 aagtcagagg ggcattggcga ggagtccagc tctgaggact ctgacattga tggccatcat  
1621 caccatcacc attaa

Reviewed and approved by site quality representative.

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