eurofins | Discovery Services

# Certificate of Analysis

## MST4, active

**(Recombinant enzyme expressed in Sf21 insect cells)**
**Item # 14-928, 14-928-K, 14-928M**
**Parent Lot # D13CP009N**

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 MST4 amino acids 4-304 expressed by baculovirus in Sf21 insect cells. Purified using $Ni^{2+}$/NTA agarose.
Purity 87% by SDS-PAGE and Coomassie blue staining. MW = 38kDa.

**Specific Activity (Parent lot# D13CP009N):** 584U/mg, where one unit of MST4, active activity is defined as 1nmol phosphate incorporated into 250µM (RLGRDKYKTLRQIRQ) per minute at 30°C with a final ATP concentration of 100µM.

**Formulation: 1.510mg/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.

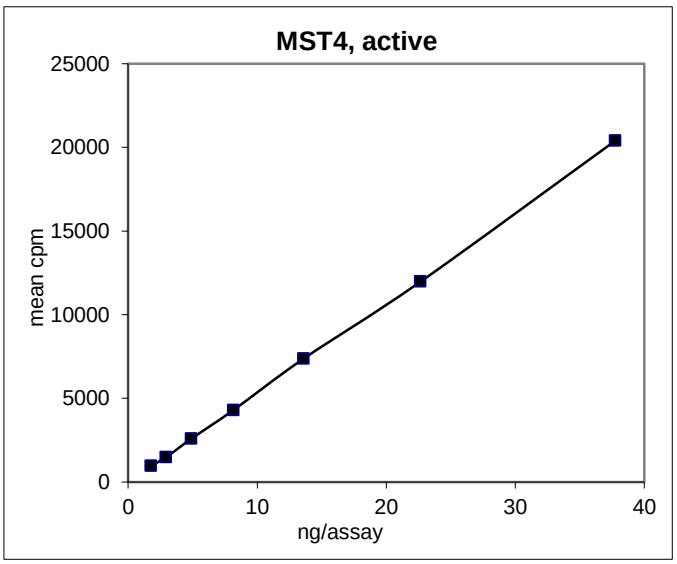
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**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.

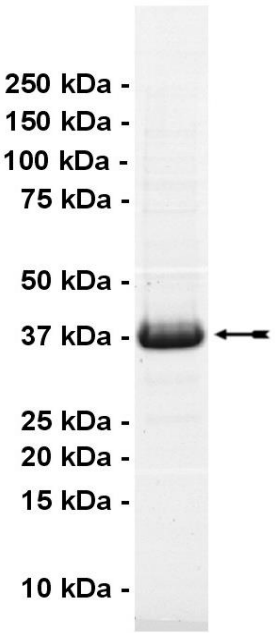
**FOR IN VITRO RESEARCH USE ONLY**
**NOT FOR USE IN HUMANS OR ANIMALS**

## Quality Control Testing

Kinase Assay: 2–38ng 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 MST4 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 MST4, active.

**Eurofins Pharma Discovery Services UK Limited**
Gemini Crescent
Dundee Technology Park
DUNDEE
DD2 1SW United Kingdom
T | +44 (0)1382 561600
F | +44 (0)1382 561601
www.eurofins.com/pharmadiscovery

eurofins | Discovery Services

# Certificate of Analysis

**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 250µM. Prepare a 2.5mM stock and add 2.5µl of stock per assay point.
3. **MST4, 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 2–38ng per assay point.
4. **[γ-$^{33}$P]ATP:** 2.5 x MgAc/[γ$^{33}$P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ$^{33}$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 **(RLGRDKYKTLRQIRQ)**.
3. Add **2.5µl (2–38ng) MST4, active.**
4. Add 5µl of $dH_2O$.
5. Add 10µl of diluted [γ-$^{33}$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.

eurofins | Discovery Services

# Certificate of Analysis

## MST4 Sequence Information

**Protein** human MST4

**Tags** *N*-terminal 6His

**Native sequence** S31 of the recombinant protein is equivalent to S4 of human MST4

**Accession number** GenBank NM_016542

***Recombinant MST4 amino acid sequence:***

```
  1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF SPVAVQVPGM QNNIADPEEL FTKLERIGKG
 61 SFGEVFKGID NRTQQVVAIK IIDLEEAEDE IEDIQQEITV LSQCDSSYVT KYYGSYLKGS
121 KLWIIMEYLG GGSALDLLRA GPFDEFQIAT MLKEILKGLD YLHSEKKIHR DIKAANVLLS
181 EQGDVKLADF GVAGQLTDTQ IKRNTFVGTP FWMAPEVIQQ SAYDSKADIW SLGITAIELA
241 KGEPPNSDMH PMRVLFLIPK NNPPTLVGDF TKSFKEFIDA CLNKDPSFRP TAKELLKHKF
301 IVKNSKKTSY LTELIDRFKR WKAEGHSDDE S
```

***Recombinant MST4 nucleotide sequence:***

```
  1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
 61 tattttcagg gcgccatgga tccggaattc tcgccggtgg ctgtccaagt gcctgggatg
121 cagaataaca tagctgatcc agaagaactg ttcacaaaat tagagcgcat tgggaaaggc
181 tcatttgggg aagttttcaa aggaattgat aaccgtaccc agcaagtcgt tgctattaaa
241 atcatagacc ttgaggaagc cgaagatgaa atagaagaca ttcagcaaga aataactgtc
301 ttgagtcaat gtgacagctc atatgtaaca aaatactatg ggtcatattt aaaggggtct
361 aaattatgga taataatgga atacctgggc ggtggttcag cactggatct tcttcgagct
421 ggtccatttg atgagttcca gattgctacc atgctaaagg aaattttaaa aggtctggac
481 tatctgcatt cagaaaagaa aattcaccga gacataaaag ctgccaatgt cttgctctca
541 gaacaaggag atgttaaact tgctgatttt ggagttgctg gtcagctgac agatacacag
601 attaaaagaa ataccttttgt gggaactcca ttttggatgg ctcctgaagt tattcaacag
661 tcagcttatg actcaaaagc tgacatttgg tcattgggaa ttactgctat tgaactagcc
721 aagggagagc cacctaactc cgatatgcat ccaatgagag ttctgtttct tattcccaaa
781 aacaatcctc caactcttgt tggagacttt actaagtctt ttaaggagtt tattgatgct
841 tgcctgaaca aagatccatc atttcgtcct acagcaaaag aacttctgaa acacaaattc
901 attgtaaaaa attcaaagaa gacttcttat ctgactgaac tgatagatcg ttttaagaga
961 tggaaggcag aaggacacag tgatgatgaa tcttaa
```

Reviewed and approved by site quality representative.