

Certificate of Analysis

Mer, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-728, 14-728-K, 14-728M

Parent Lot # 32932U

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, recombinant, human Mer, amino acids 557–882, expressed by baculovirus in Sf21 insect cells. Purified using glutathione-agarose. Purity 74% by SDS-PAGE and Coomassie blue staining. MW = 64.4kDa.

Specific Activity (Parent lot# 32932U): 9262U/mg, where one unit of Mer, active activity is defined as 1nmol phosphate incorporated into 250µM (GGMEDYFEFMGGKKK) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.38mg/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.

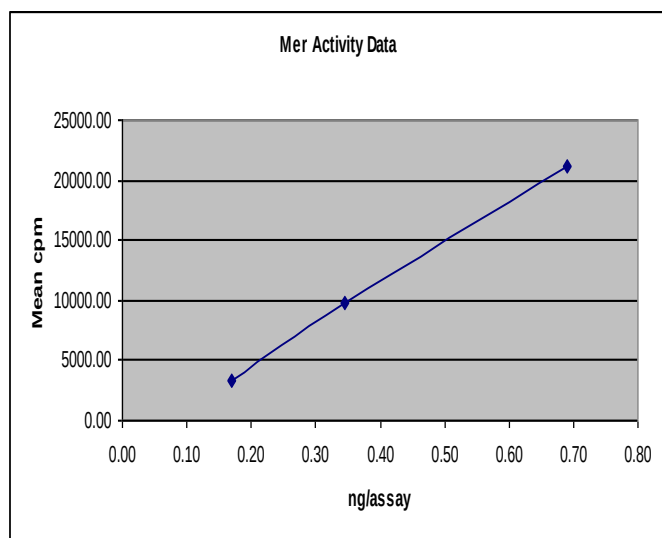
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

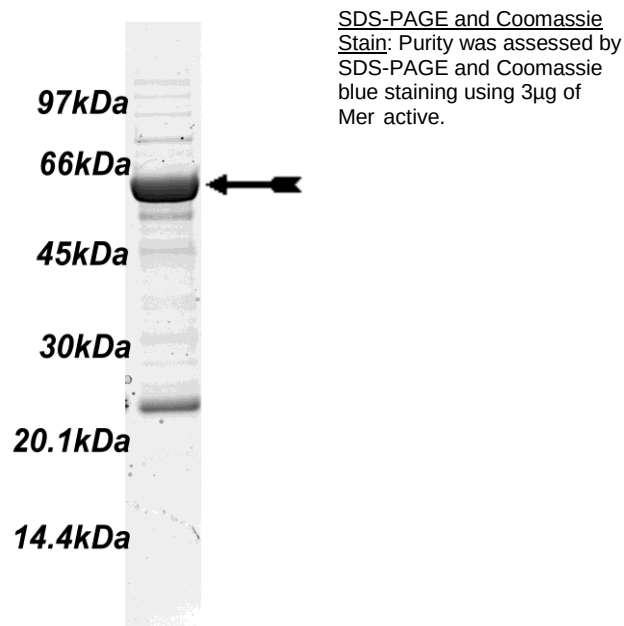
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 0.17–0.69ng of this lot of enzyme phosphorylated 250µM (GGMEDYFEFMGGKKK) 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 Mer 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. **(GGMEDIYFEFMGGKKK):** Use at a final assay concentration of 250µM. Make up a 2.5mM stock. Add 2.5µl of stock per assay point.
3. **NaCl:** Use at a final assay concentration of 30mM. Make a 3M stock. Add 0.25µl of stock per assay point.
4. **Mer, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.17–0.69ng per assay point.
5. **[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 **(GGMEDIYFEFMGGKKK)**.
3. Add **2.5µl (0.17–0.69ng) Mer active**.
4. Add 0.25µl of 3M NaCl.
5. Add 4.75µl of dH₂O.
6. Add 10µl of diluted [γ -³³P]ATP mixture.
7. Incubate for 10 minutes at 30°C.
8. Stop the reaction by adding 5µl of 3% phosphoric acid.
9. Transfer a 10µl 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 1µl of 30% phosphoric acid.

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Mer Sequence Information

<u>Protein</u>	Human Mer
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	R231 of recombinant sequence is equivalent to R557 of native human Mer
<u>Accession number</u>	GenBank NM_006343. The recombinant protein contains the amino acid substitutions H628Q and R794A with reference to NM_006343. H628Q is reported in GenBank AA398845 and BG566419; R794A is reported in GenBank BG109051 and BX280572.

Recombinant Mer amino acid sequence:

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1  MSPILGYWKI  KGLVQPTRL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQSMA  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121  DFLSKLPEML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181  KRIEAIQID  KYLKSSKYIA  WPLQGWQATF  GGGDHPKSD  LEVLFQGPEF  RRAIELTLHS
241  LGVSEELQNK  LEDVVIDRNL  LILGKILGEG  EFGSVMEGNL  KQEDGTSCLK  AVKTMKLDNS
301  SQREIEEFSL  EAACMKDFSH  PNVIRLLGVC  IEMSSQGIPK  PMVILPFMKY  GDLHTYLLYS
361  RLETGPKHIP  LQTLLKFMVD  IALGMEYLSN  RNFLHRDLAA  RNCMLRDDMT  VCVADFGLSK
421  KIYSGDYRQ  GRIAKMPVKW  IAIESLADRV  YTSKSDVWAF  GVTMWEIATR  GMTYPYGVQN
481  HEMYDYLLHG  HRLKQPEDCL  DELYEIMYSC  WRTDPLDRPT  FSVLRLQLEK  LLESPLDVRN
541  QADVIVNTQ  LLESSE

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Recombinant Mer nucleotide sequence:

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1  atgtccccta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tcgacttctt
61  ttggaatata  ttgaagaaaa  atatgaagag  catttgtatg  agcgcgatga  aggtgataaa
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481  gttgttttat  acatggaccc  aatgtgcctg  gatgcgttcc  caaaattagt  ttgttttaaa
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1321  atcgccatag  aaagtcttgc  agaccgagtc  tacacaagta  aaagtgatgt  gtgggcattt
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1501  gatgaactgt  atgaaataat  gtactcttgc  tggagaaccg  atcccttaga  ccgccccacc
1561  ttttcagtat  tgaggctgca  gctagaaaaa  ctcttagaaa  gtttgctgta  cgttcggaac

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1621 caagcagacg ttatttacgt caatacacag ttgctggaga gctctgagta a

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