

Certificate of Analysis

Yes, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-478, 14-478-K, 14-478M

Parent Lot # 25589U

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 Yes, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose. Purity 69.6% by SDS-PAGE and Coomassie blue staining. MW = 62.7kDa.

Specific Activity (Parent lot# 25589U): 5558U/mg, where one unit of Yes, active activity is defined as 1nmol phosphate incorporated into 0.1mg/ml poly(Glu,Tyr) (4:1) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 4.505mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol. Frozen solution.

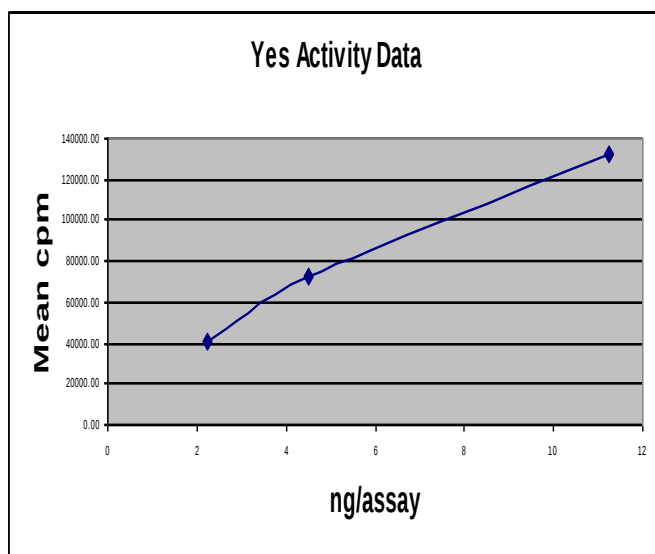
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

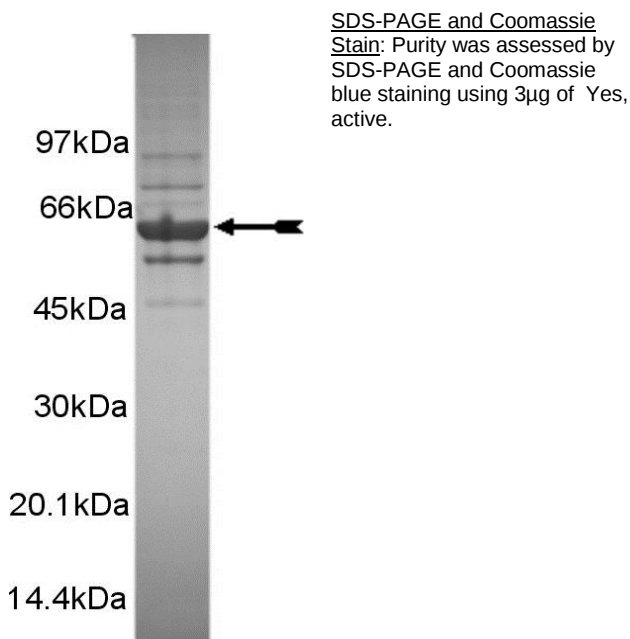
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 2.2–11.3ng of this lot of enzyme phosphorylated 0.1mg/ml poly(Glu,Tyr) (4:1) 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 Yes with the translated 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. **Yes, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 2.2–11.3ng per assay point.
3. **[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.)
4. **Poly(Glu,Tyr) (4:1):** Use a final assay concentration of 0.1mg/ml. Make a stock solution of 1mg/ml. Add 2.5 μ l of stock per assay point.

Assay Procedure (96 well plate format):

1. Add 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of **poly(Glu,Tyr) (4:1)**.
3. Add **2.5 μ l (2.2–11.3ng) Yes, active**.
4. Add 5 μ l of dH₂O.
5. Add 10 μ l of the diluted [γ -³³P] ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ l aliquot onto the appropriate area of a **Filtermat A**.
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 1 μ l of 30% phosphoric acid.

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Yes Sequence Information

<u>Protein</u>	Human Yes
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M8 of the recombinant protein is equivalent to M1 of human Yes
<u>Accession number</u>	GenBank M15990

Recombinant Yes amino acid sequence:

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1  MHHHHHHMGC  IKSKENKSPA  IKYRPENTPE  PVSTSVSHYG  AEPTTVSPCP  SSSAKGTAVN
61  FSSLSMTPFG  GSSGVTPFGG  ASSSFVSVPS  SYPAGLTGGV  TIFVALYDYE  ARTTEDLSFK
121 KGERFQIINN  TEGDWWEARS  IATGKNGYIP  SNYVAPADSI  QAEEWYFGKM  GRKDAERLLL
181 NPGNQRGIFL  VRESETTKGA  YSLSIRDWDE  IRGDNVKHYK  IRKLDNGGY  ITTRAQFDL
241 QKLVKHYTEH  ADGLCHKLTT  VCPTVKPQTQ  GLAKDAWEIP  RESLRLEVKL  GQGCFGEVWM
301 GTWNGTTKVA  IKTLKPGTMM  PEAFLQEAQI  MKKL RHDKLV  PLYAVVSEEP  IYIVTEFMSK
361 GSLLDFLKEG  DGKYLKLPQL  VDMAAQIADG  MAYIERMNYI  HRDLRAANIL  VGENLVCKIA
421 DFGLARLIED  NEYTARQGAK  FPIKWTAPEA  ALYGRFTIKS  DVWSFGILQT  ELVTKGRVPY
481 PGMVNREVLE  QVERGYRMPC  PQGCPESLHE  LMNLCWKKDP  DERPTFEYIQ  SFLEDYFTAT
541 EPQYQPGENL

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Recombinant Yes nucleotide sequence:

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1  atgcatcacc  atcaccatca  tatgggctgc  attaaaagta  aagaaaacaa  aagtccagcc
61  attaaatata  gacctgaaaa  tactccagag  cctgtcagta  caagtgtgag  ccattatgga
121 gcagaaccca  ctacagtgtc  accatgtccg  tcatcttcag  caaaggggaa  agcagttaat
181 ttcagcagtc  tttccatgac  accatttgga  ggatcctcag  gggtaacgcc  ttttgagggt
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1621 gagccacagt  accagccagg  agaaaattta  taa

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