

## Certificate of Analysis

### JAK1, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-918, 14-918-K, 14-918M

Parent Lot # D12KP003N

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 JAK1 amino acids 866-end, expressed by baculovirus in Sf21 insect cells. Purified using glutathione sepharose. Activated *in vitro* by auto-phosphorylation.

Purity 76% by SDS-PAGE and Coomassie blue staining. MW = 61kDa.

**Specific Activity (Parent lot# D12KP003N):** 76U/mg, where one unit of JAK1, active activity is defined as 1nmol phosphate incorporated into 500  $\mu$ M (GEEPLYWSFPAKKK) per minute at 30°C with a final ATP concentration of 100 $\mu$ M.

**Formulation:** 0.623mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 5mM  $\beta$ -glycerophosphate, 0.5mM sodium orthovanadate, 50% v/v glycerol, 0.02% Triton® X-100, 0.5mM EDTA, 2mM DTT. Frozen solution.

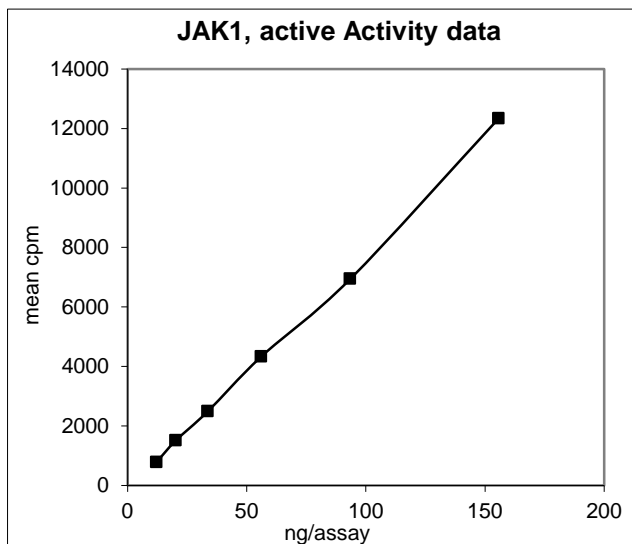
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**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.

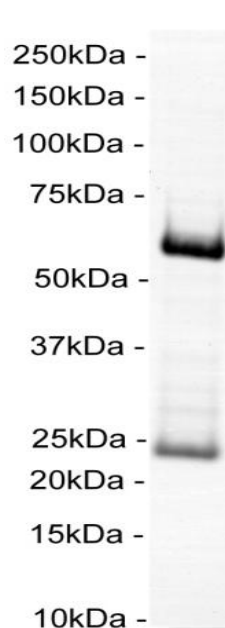
**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### Quality Control Testing

**Kinase Assay:** 12–156ng of this lot of enzyme phosphorylated 500 $\mu$ M (GEEPLYWSFPAKKK) 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 JAK1 with the translated sequence listed on page four.



**SDS-PAGE and Coomassie Stain:** Purity was assessed by SDS-PAGE and Coomassie blue staining using 3 $\mu$ g of JAK1, active.

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### Kinase Assay Protocol

#### Stock Solutions:

1. **5 x Reaction Buffer:** 100mM Tris/HCl pH7.5, 1mM EDTA.
2. **(GEEPLYWSFPAKKK):** Use at a final assay concentration of 500  $\mu$ M. Prepare a 5mM stock and add 2.5 $\mu$ l of stock per assay point.
3. **JAK1, active:** Dilute with 20mM Tris/HCl pH7.5, 0.2mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol. Use 12–156ng per assay point.
4.  **$[\gamma\text{-}^{33}\text{P}]\text{ATP}$ :** 2.5 x MgAc/ $[\gamma\text{-}^{33}\text{P}]\text{ATP}$  cocktail: 25mM MgAc and 0.25mM ATP to which is added  $[\gamma\text{-}^{33}\text{P}]\text{ATP}$  (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 **(GEEPLYWSFPAKKK)**.
3. Add **2.5  $\mu$ l (12–156ng) JAK1, active**.
4. Add 5  $\mu$ l of dH<sub>2</sub>O.
5. Add 10 $\mu$ l of diluted  $[\gamma\text{-}^{33}\text{P}]\text{ATP}$  mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 $\mu$ l of 3% phosphoric acid.
8. Transfer a 10 $\mu$ l 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 1 $\mu$ l of 30% phosphoric acid.

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## JAK1 Sequence Information

<b><u>Protein</u></b>	Human JAK1
<b><u>Tags</u></b>	N-terminal GST
<b><u>Native sequence</u></b>	D238 of the recombinant protein is equivalent to D866 of human JAK1
<b><u>Accession number</u></b>	GenBank NM_002227

### Recombinant JAK1 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 KRIEAIPQID  KYLKSSKYIA  WPLQGWAQTF  GGGDHPKSD  LVPRGSQTSL  YKKAGTMDPT
241 HFEKRFLKRI  RDLGEGHFGK  VELCRYDPEG  DNTGEQVAVK  SLKPESGGNH  IADLKKEIEI
301 LRNLYHENIV  KYKGICTEDG  GNGIKLIMEF  LPSGSLKEYL  PKNKNKINLK  QQLKYAVQIC
361 KGMDYLGSRQ  YVHRDLAARN  VLVESEHQVK  IGDFGLTKAI  ETDKEYYTVK  DDRDSPVFWY
421 APECLMQSKF  YIASDVWSFG  VTLHELLTYC  DSDSSPMALF  LKMIGPTHGQ  MTVTRLVNTL
481 KEGKRLPCPP  NCPDEVYQLM  RKCWEFQPSN  RTSFQNLIEG  FEALLK

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### Recombinant JAK1 nucleotide sequence:

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1  atgtccccta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tcgacttctt
61  ttggaatata  ttgaagaaaa  atatgaagag  catttgatat  agcgcgatga  aggtgataaa
121  tggcgaaaca  aaaagtttga  attgggtttg  gagtttccca  atcttcctta  ttatatattg
181  ggtgatgtta  aattaacaca  gtctatggcc  atcatacgtt  atatagctga  caagcacaac
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421  acatatttaa  atgggtgatca  tgtaaccat  cctgacttca  tgttgatga  cgctcttgat
481  gttgttttat  acatggacce  aatgtgcctg  gatgcgttcc  caaaattagt  ttgttttaa
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901  ttaaggaacc  tctatcatga  gaacattgtg  aagtacaaag  gaatctgac  agaagacgga
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1201 gaaaccgata  aggagtatta  caccgtcaag  gatgaccggg  acagccctgt  gttttggtat
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1561 tttgaagcac  ttttaaaata  a

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