

## Certificate of Analysis

### JAK3, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-629, 14-629-K, 14-629M

Parent Lot # D8EN006U

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 JAK3 amino acids 781–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni<sup>2+</sup>-NTA agarose. Purity 44% by SDS-PAGE and Coomassie blue staining. MW = 39.9kDa.

**Specific Activity (Parent lot# D8EN006U):** 1218U/mg, where one unit of JAK3, active activity is defined as 1nmol phosphate incorporated into 500µM (GGESEYFELVKKKK) per minute at 30°C with a final ATP concentration of 100µM.

**Formulation:** 0.473mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 20mM β-glycerophosphate, 0.25mM sodium orthovanadate, 10mM NaF, 0.1mM EGTA, 0.03% Brij-35, 10% glycerol, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

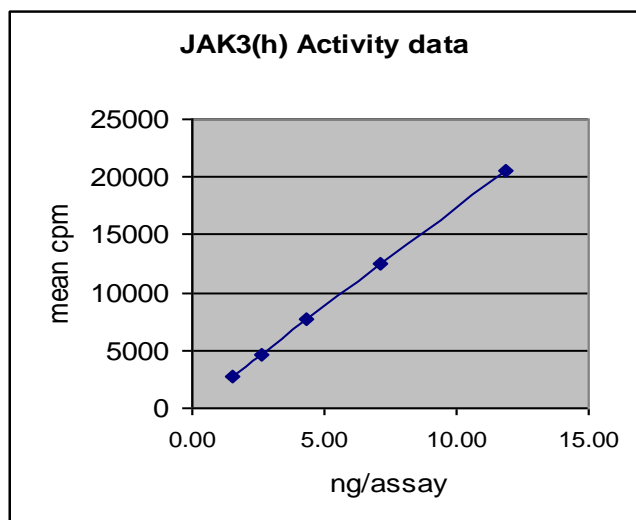
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**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.

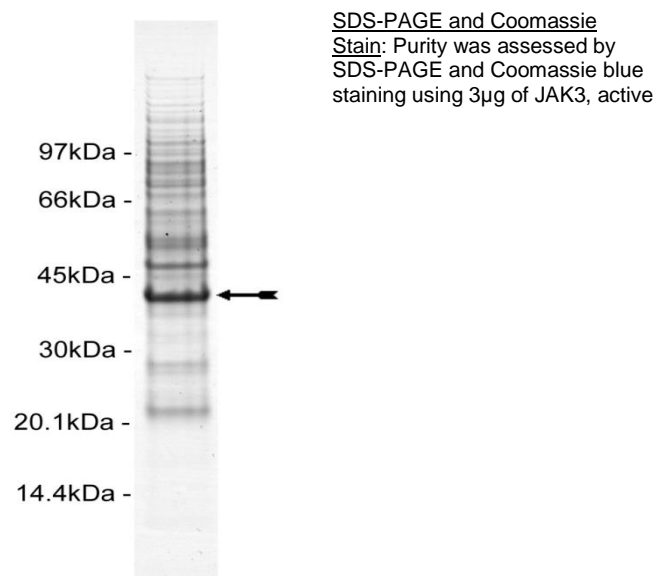
**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### Quality Control Testing

**Kinase Assay:** 1.5–11.0ng of this lot of enzyme phosphorylated 500µM (GGESEYFELVKKKK) 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 JAK3 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. **(GGEEEEYFELVKKKK):** 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. **JAK3, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1.5–11.0ng per assay point.
4. **[ $\gamma$ -<sup>33</sup>P]ATP:** 2.5 x magnesium acetate/[ $\gamma$ -<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 **(GGEEEEYFELVKKKK)**.
3. Add **2.5 $\mu$ l (1.5–11.0ng) JAK3, active**.
4. Add 5 $\mu$ l of dH<sub>2</sub>O.
5. Add 10 $\mu$ l of diluted [ $\gamma$ -<sup>33</sup>P]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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## JAK3 Sequence Information

<b><u>Protein</u></b>	human JAK3
<b><u>Tags</u></b>	N-terminal 6His
<b><u>Native sequence</u></b>	I8 of recombinant sequence is equivalent to I781 of native human JAK3
<b><u>Accession number</u></b>	GenBank AF513860

### Recombinant JAK3 amino acid sequence:

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1 MHHHHHHISS DYELLSDPTP GALAPRDGLW NGAQLYACQD PTIFEERHLK YISQLGKGNF
61 GSVELCRYDP LGDNTGALVA VKQLQHSQPD QQRDFQREIQ ILKALHSDFI VKYRGVSYGP
121 GRQSLRLVME YLPSGCLRDF LQRHRARLDA SRLLLYSSQI CKGMEYLGSR RCVHRDLAAR
181 NILVESEAHV KIDAFGLAKL LPLDKDYVVV REPGQSPIFW YAPESLSDNI FSRQSDVWSF
241 GVVLYELFTY CDKSCSPSAE FLRMMGCERD VPALCRLELE LEEGQRLPAP PACPAEVHEL
301 MKLCWAPSPQ DRPSFSALGP QLDMLWSGSR GCETHAFTAH PEGKHHSLSF S

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### Recombinant JAK3 nucleotide sequence:

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1 atgcatcatc accatcacca tatctcttca gactatgagc tcctctcaga cccacacact
61 ggtgccctgg cacctcgtga tgggctgtgg aatggtgcc agctctatgc ctgccaagac
121 cccacgatct tcgaggagag acacctcaag tacatctcac agctgggcaa gggcaacttt
181 ggagcgtgg agctgtgcc ctatgacccg ctaggcgaca atacaggtgc cctggtggcc
241 gtgaaacagc tgcagcacag cgggcccagac cagcagaggg actttcagcg ggagattcag
301 atcctcaaag cactgcacag tgatttcatt gtcaagtatc gtggtgtcag ctatggcccc
361 ggccgccaga gcctgcggct ggtcatggag tacctgcccc gggctgctt gcgcgacttc
421 ctgcagcggc accgcgcgcg cctcgatgcc agccgcctcc ttctctattc ctgcagatc
481 tgcaagggca tggagtacct gggctcccgc cgctgcgtgc accgcgacct ggccgccgca
541 aacatcctcg tggagagcga ggcacacgtc aagatcgctg acttcggcct agctaagctg
601 ctgccgcttg acaaagacta ctacgtggtc cgcgagccag gccagagccc cttttctgtg
661 tatgcccccg aatccctctc ggacaacatc ttctctcgcc agtcagacgt ctggagcttc
721 ggggtcgtcc tgtacgagct cttcacctac tgcgacaaaa gctgcagccc ctgcccag
781 ttctgcgga tgatgggatg tgagcgggat gtccccgcc tctgccgctt cttggaactg
841 ctggaggagg gccagaggct gccggcgcct cctgcctgcc ctgctgaggt tcacgagctc
901 atgaagctgt gctgggcccc tagcccacag gaccggccat cattcagcgc cctgggcccc
961 cagctggaca tgctgtggag cggagccgg ggggtgtgaga ctcatgcctt cactgctcac
1021 ccagagggca aacaccactc cctgtccttt tcatag

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