

Certificate of Analysis

JAK2, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-640, 14-640-K, 14-640M

Parent Lot # 1919213

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: C-terminal 6His-tagged, recombinant, human JAK2, amino acids 808–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose followed by gel filtration. Purity 55.2% by SDS-PAGE and Coomassie blue staining. MW = 38.9kDa.

Formulation: 0.509mg/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.

Specific Activity (Parent lot# 1919213): 3627U/mg, where one unit of JAK2, active activity is defined as 1nmol phosphate incorporated into 100µM PDKtide per minute at 30°C with a final ATP concentration of 100µM.

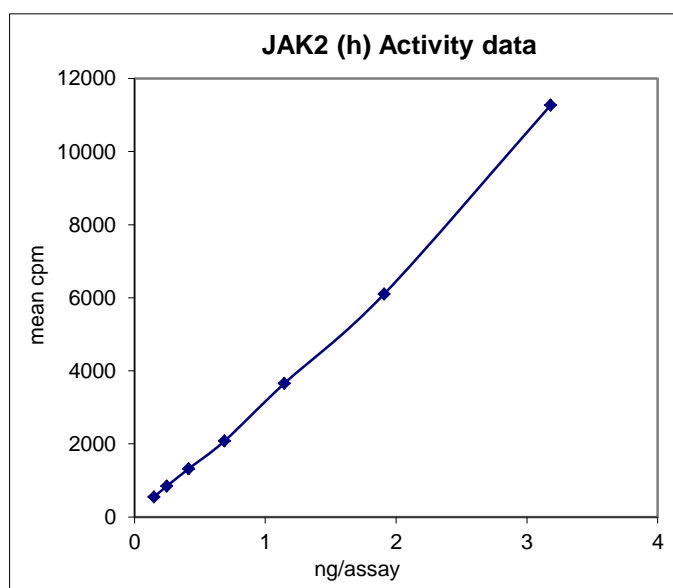
Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

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

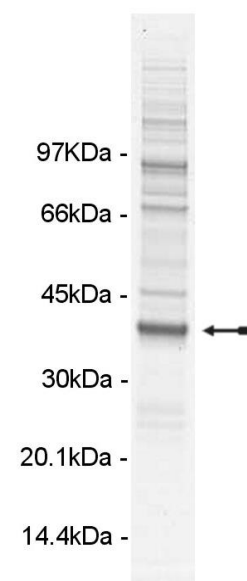
Quality Control Testing

Kinase Assay: 0.2–3.2ng of this lot of enzyme phosphorylated 100µM PDKtide 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 JAK2 with the translated native sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of JAK2, active.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **PDKtide (KTFCGTPEYLAPEVRREPRILSEEEQEMFRDFD YIADWC):** Use at a final assay concentration of 100 μ M. Prepare a 1mM stock. Add 2.5 μ l of stock per assay point.
3. **JAK2, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.2–3.2ng per assay point.
4. **[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 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of **PDKtide (KTFCGTPEYLAPEVRREPRILSEEEQEMFRDFDYIADWC)**.
3. Add **2.5 μ l (0.2–3.2ng) JAK2, active**.
4. Add 5 μ l of dH₂O.
5. Add 10 μ l of 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 **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 μ l of 30% phosphoric acid.

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JAK2 Sequence Information

<u>Protein</u>	human JAK2
<u>Tags</u>	C-terminal 6His
<u>Native sequence</u>	L2 of recombinant sequence is equivalent to L808 of native human JAK2
<u>Accession number</u>	GenBank NM_004972

Recombinant JAK2 amino acid sequence:

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1 MLFTPDYELL TENDMLPNMR IGALGFSGAF EDRDPTQFEE RHLKFLQQLG KGNFGSVEMC
61 RYDPLQDNTG EVVAVKKLQH STEEHLRDFE REIEILKSLQ HDNIVKYKGV CYSAGRRNLK
121 LIMEYLPYGS LRDYLQKHKE RIDHIKLLQY TSQICKGMEY LGTKRYIHRD LATRNILVEN
181 ENRVKIGDFG LTKVLPQDKE YYKVKEPGES PIFWYAPESL TESKFSVASD VWSFGVVLYE
241 LFTYIEKSKS PPAEFMRMIG NDKQGQMIVF HLIPELLKNNG RLPRPDGCPD EIYMIMTECW
301 NNNVNQRPSF RDLALRVDQI RDNMAGHHHH HH
  
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Recombinant JAK2 nucleotide sequence:

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1 atgttgttta ctccagatta tgaactatta acagaaaatg acatgttacc aaatatgagg
61 ataggtgccc taggtgtttc tgggtgcctt gaagaccggg atcctacaca gtttgaagag
121 agacatttga aatttctaca gcaacttggc aagggttaatt ttgggagtggt ggagatgtgc
181 cggtatgacc ctctacagga caaacttggg gaggtggtcg ctgtaaaaaa gcttcagcat
241 agtactgaag agcacctaag agactttgaa agggaaattg aaatcctgaa atccctacag
301 catgacaaca ttgtaaagta caaggagtg tgctacagtg ctggtcggcg taatctaaaa
361 ttaattatgg aatatttacc atatggaagt ttacgagact atcttcaaaa acataaagaa
421 cggatagatc acataaaaact tctgcagtac acatctcaga tatgcaaggg tatggagtat
481 cttggtacaa aaaggtatat ccacagggat ctggcaacga gaaatatatt ggtggagaac
541 gagaacagag ttaaaattgg agattttggg ttaaccaaaag tcttgccaca agacaaaagaa
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