eurofins | Discovery Services

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^{2+}-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 (GGEEEEYFELVKKKK) 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.

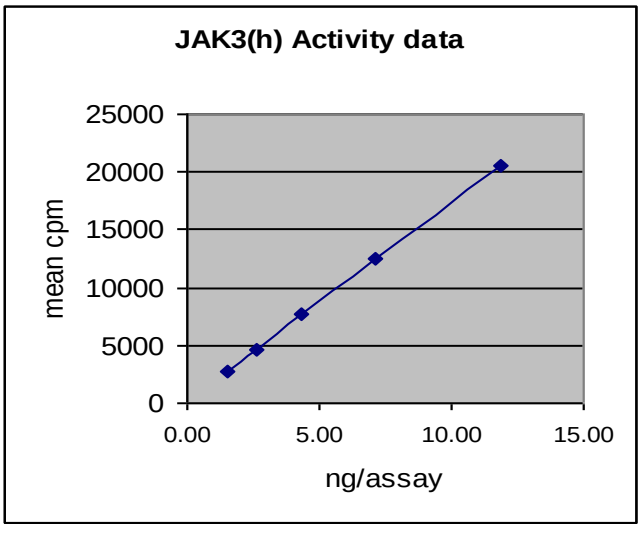
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

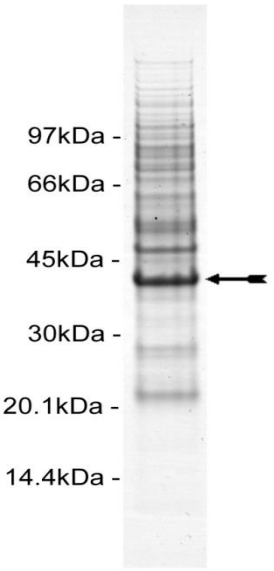
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 1.5–11.0ng of this lot of enzyme phosphorylated 500µM (GGEEEEYFELVKKKK) 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.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of JAK3, active

Eurofins Pharma Discovery Services UK Limited
Gemini Crescent
Dundee Technology Park
DUNDEE
DD2 1SW United Kingdom
T | +44 (0)1382 561600
F | +44 (0)1382 561601
www.eurofins.com/pharmadiscovery

eurofins | Discovery Services

Certificate of Analysis

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 500µM. Prepare a 5mM stock and add 2.5µl 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. **[γ-^{33}P]ATP:** 2.5 x magnesium acetate/[γ-^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-^{33}P]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 **(GGEEEEYFELVKKKK)**.
3. Add **2.5µl (1.5–11.0ng) JAK3, active**.
4. Add 5µl of dH_2O.
5. Add 10µl of diluted [γ-^{33}P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl 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 1µl of 30% phosphoric acid.

eurofins | Discovery Services

Certificate of Analysis

JAK3 Sequence Information

Protein human JAK3

Tags *N*-terminal 6His

Native sequence I8 of recombinant sequence is equivalent to I781 of native human JAK3

Accession number GenBank AF513860

Recombinant JAK3 amino acid sequence:

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

Recombinant JAK3 nucleotide sequence:

```
   1 atgcatcatc accatcacca tatctcttca gactatgagc tcctctcaga ccccacacct
  61 ggtgccctgg cacctcgtga tgggctgtgg aatggtgccc agctctatgc ctgccaagac
 121 cccacgatct tcgaggagag acacctcaag tacatctcac agctgggcaa gggcaacttt
 181 ggcagcgtgg agctgtgccg ctatgacccg ctaggcgaca atacaggtgc cctggtggcc
 241 gtgaaacagc tgcagcacag cgggccagac cagcagaggg actttcagcg ggagattcag
 301 atcctcaaag cactgcacag tgatttcatt gtcaagtatc gtggtgtcag ctatggcccg
 361 ggccgccaga gcctgcggct ggtcatggag tacctgccca gcggctgctt gcgcgacttc
 421 ctgcagcggc accgcgcgcg cctcgatgcc agccgcctcc ttctctattc ctcgcagatc
 481 tgcaagggca tggagtacct gggctcccgc cgctgcgtgc accgcgacct ggccgcccga
 541 aacatcctcg tggagagcga ggcacacgtc aagatcgctg acttcggcct agctaagctg
 601 ctgccgcttg acaaagacta ctacgtggtc cgcgagccag gccagagccc cattttctgg
 661 tatgcccccg aatccctctc ggacaacatc ttctctcgcc agtcagacgt ctggagcttc
 721 ggggtcgtcc tgtacgagct cttcacctac tgcgacaaaa gctgcagccc ctcggccgag
 781 ttcctgcgga tgatgggatg tgagcgggat gtccccgccc tctgccgcct cttggaactg
 841 ctggaggagg gccagaggct gccggcgcct cctgcctgcc ctgctgaggt tcacgagctc
 901 atgaagctgt gctgggcccc tagcccacag gaccggccat cattcagcgc cctgggcccc
 961 cagctggaca tgctgtggag cggaagccgg gggtgtgaga ctcatgcctt cactgctcac
1021 ccagagggca aacaccactc cctgtccttt tcatag
```

Reviewed and approved by site quality representative.