

Certificate of Analysis

TYK2, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-924, 14-924-K, 14-924M

Parent Lot # D14BP001N

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 TYK2 amino acids 875-end, expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography followed by gel filtration.

Purity 78.5% by SDS-PAGE and Coomassie blue staining. MW = 40kDa.

Specific Activity (Parent lot# D14BP001N): 65U/mg, where one unit of TYK2, active activity is defined as 1nmol phosphate incorporated into 250 μ M (GGMEDIYFEFMGGKKK) per minute at 30°C with a final ATP concentration of 100 μ M.

Formulation: 0.175mg/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.

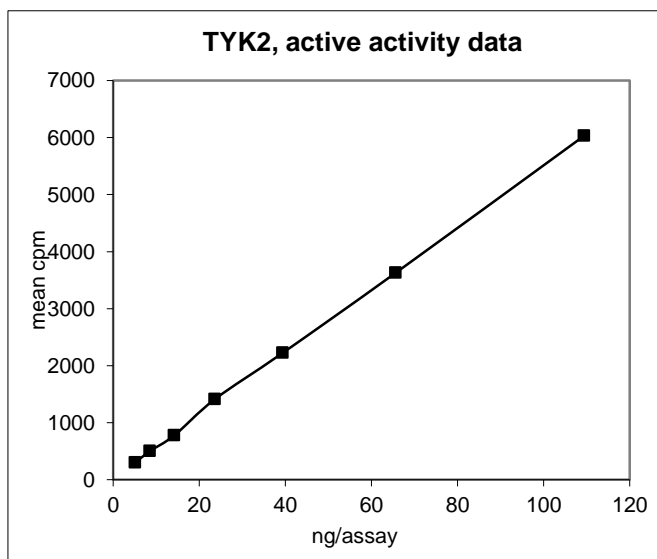
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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**

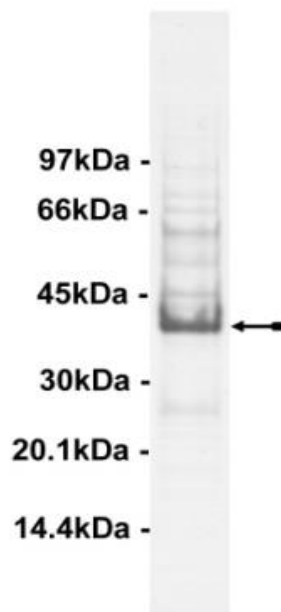
Quality Control Testing

Kinase Assay: 5–109ng of this lot of enzyme phosphorylated 250 μ M (GGMEDIYFEFMGGKKK) 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 TYK2 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 μ g of TYK2, active.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **(GGMEDIYFEFMGGKKK):** Use at a final assay concentration of 250 μ M. Prepare a 2.5mM stock and add 2.5 μ l of stock per assay point.
3. **TYK2, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 5–109ng per assay point.
4. **[γ -³³P]ATP:** 2.5 x MgAc/[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 **(GGMEDIYFEFMGGKKK)**.
3. Add **2.5 μ l (5–109ng) TYK2, 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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TYK2 Sequence Information

| | |
|--------------------------------|--|
| <u>Protein</u> | Human TYK2 |
| <u>Tags</u> | N-terminal 6His |
| <u>Native sequence</u> | A31 of the recombinant protein is equivalent to A875 of human TYK2 |
| <u>Accession number</u> | GenBank NM_003331 |

Recombinant TYK2 amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF ADVLTVNPDS PASDPTVFHK RYLKKIRDLD
61 EGHFGKVS LY CYDPTNDGTG EMVAVKALKA DCGPQHRSGW KQEIDILRTL YHEHI IKYKG
121 CCEDQGEKSL QLVMEYVPLG SLRDYLPRHS IGLAQLLLFA QQICEGMAYL HSQHYIHRDL
181 AARNVLLDND RLVKIGDFGL AKAVPEGHEY YRVREDGDSP VFWYAPECLK EYKFYYASDV
241 WSGVTLYEL LTHCDSSQSP PTKFLELIGI AQQQMTVLRL TELLERGERL PRPDKCPCEV
301 YHLMKNCWET EASFRPTFEN LIPILKTVHE KYQQQAPSVF SVC
  
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Recombinant TYK2 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tccaacgac cgaaaacctg
61 tatttttcagg gcgccatgga tccggaattc gctgacgtct tgactgtgaa cccggactca
121 ccggcgtcgg accctacggg tttccacaag cgctatttga aaaagatccg agatctgggc
181 gagggctcact tcggcaaggc cagcttgtag tgctacgacg cgaccaacga cggcactggc
241 gagatgggtgg cggtgaaagc cctcaaggca gactgcggcc cccagcaccg ctcgggctgg
301 aagcaggaga ttgacattct gcgcacgctc taccacgagc acatcatcaa gtacaagggc
361 tgctgcgagg accaaggcga gaagtcgctg cagctgggca tggagtacgt gccctggg
421 agcctccgag actacctgcc ccggcacagc atcgggctgg cccagctgct gctcttcgcc
481 cagcagatct gcgagggcat ggcctatctg cactcgcagc actacatcca ccgagacct
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1021 agcgtgtgct ga
  
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