

Certificate of Analysis

IRAK4, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-599, 14-599-K, 14-599M

Parent Lot # 2463698

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 full length, human IRAK4, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose. Purity 81.4% by SDS-PAGE and Coomassie blue staining. MW = 55.4kDa.

Specific Activity (Parent lot# 2463698): 1818U/mg, where one unit of IRAK4, active activity is defined as 1nmol phosphate incorporated into 0.33mg/ml myelin basic protein per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.874mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol. Frozen solution.

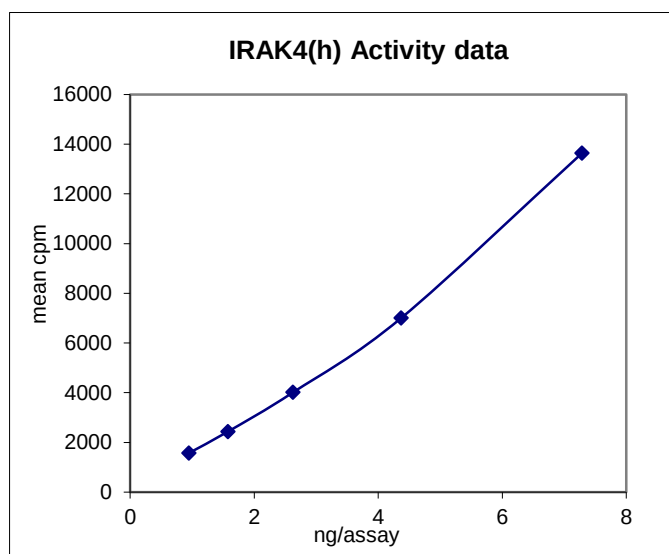
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

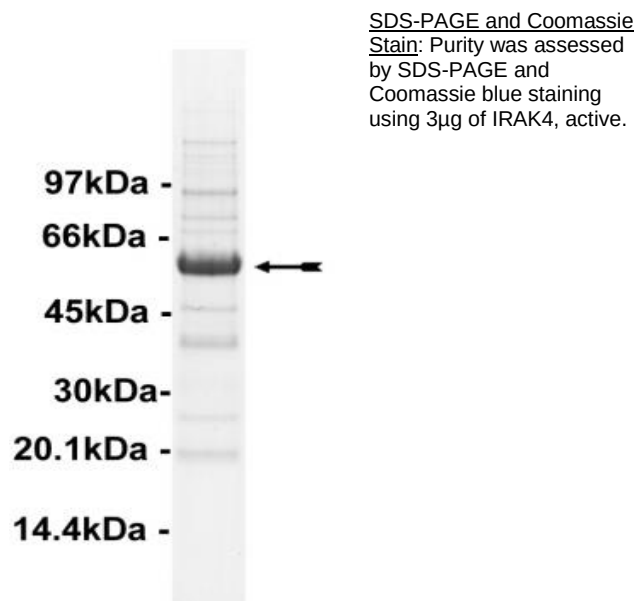
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 0.9–7.3ng of this lot of enzyme phosphorylated 0.33mg/ml myelin basic protein (MBP) 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 IRAK4 with the translated 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. Myelin Basic Protein (MBP):** Use at a final assay concentration of 0.33mg/ml. Make up a 3.3mg/ml stock. Use 2.5µl of stock per assay point.
- 3. IRAK4, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.9–7.3ng 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 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **myelin basic protein (MBP)**.
3. Add **2.5µl (0.9–7.3ng) IRAK4, active**.
4. Add 5µl of dH₂O.
5. Add 10µl of diluted [γ -³³P]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.

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IRAK4 Sequence Information

Protein	Human IRAK4
Tags	N-Terminal 6His
Native sequence	M31 of the fusion protein is equivalent to M1 of native human IRAK4
Accession number	GenBank AF445802

Recombinant IRAK4 amino acid sequence:

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1 MSYYHHHHH DYDIPTTENL YFQGAMDPEF MNKPITPSTY VRCLNVGLIR KLSDFIDPQE
61 GWKKLAVAIK KPSGDDRYNQ FHIRRFEALL QTGKSPTSEL LFDWGTTNCT VGDLVDLLIQ
121 NEFFAPASLL LPDAVPKTAN TLPSKEAITV QQKQMPFCDK DRTLMTVPQN LEQSYMPDPS
181 SSPENKSLEV SDTRFHSFSF YELKNVTNNF DERPISVGGN KMGEFGFVV YKGYVNNTTV
241 AVKKLAAMVD ITTEELKQQF DQEIYMAKC QHENLVELLG FSSDGDCLCL VYVYMPNGSL
301 LDRLSCLDGT PPLSWHMRCK IAQGAANGIN FLHENHHIHR DIKSANILLD EAFTAKISDF
361 GLARASEKFA QVTMTSRIVG TTAYMAPEAL RGEITPKSDI YSFGVLLLEI ITGLPAVDEH
421 REPQLLLDIK EEIEDEEKTI EDYIDKKMND ADSTSVEAMY SVASQCLHEK KNKRDPDIKKV
481 QQLLQEMTAS

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Recombinant IRAK4 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatgga tccggaattc atgaacaaac ccataacacc atcaacatat
121 gtgcgctgcc tcaatgttgg actaattagg aagctgtcag attttattga tcctcaagaa
181 ggatggaaga agttagctgt agctattaaa aaaccatctg gtgatgatag atacaatcag
241 ttccacataa ggagatttga agcattactt caaactggaa aaagtccac ttctgaatta
301 ctgtttgact ggggcaccac aaattgcaca gttggtgatc ttgtggatct tttgatccaa
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1441 caacagctgc tgcaagagat gacagcttct taa

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