Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ IRAK4 (untagged)

interleukin-1 receptor associated kinase 4

Recombinant Human Active Protein Kinase

HGNC Symbol: IRAK4

Synonyms: REN64; NY-REN-64; IPD1

Product No.: 0586-0000-1

Lot: 006

Description: Human IRAK4, C-terminal fragment, amino acids A_{104}-S_{460} (as in NCBI/Protein entry NP_057207.2), untagged, expressed in Sf9 insect cells

Product identity: IRAK4 Lot 006, was confirmed as IRAK4 by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 40,378 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

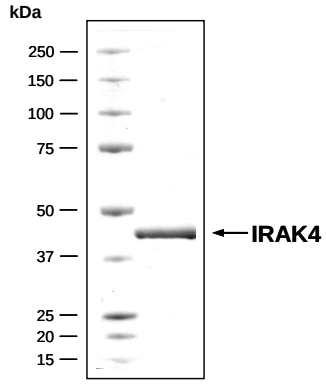
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 20 % glycerol

Storage temperature: -80°C
For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10µl are not advisable. Avoid repeated freeze-thaw cycles!

Protein concentration: 0.119 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

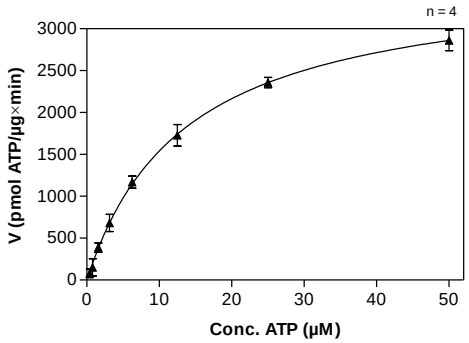
Biochemical Parameters:
Specific kinase activity (P_i transfer): 3640 pmol/µg × min
ATP-K_M: 14 µM

IRAK4 Lot 006:
Coomassie stain



2.0 µg IRAK4

IRAK4 Lot 006:
Determination of V_{max} and K_M value for ATP



Determination of K_M value & Specific activity:
• Assay conditions:
60 mM HEPES-NaOH, pH 7.5
3 mM $MgCl_2$
3 mM $MnCl_2$
3 µM Na-orthovanadate
1.2 mM DTT
50 µg/ml $PEG_{20.000}$
ATP (variable)
Substrate: Histone H2B 100 µg/ml
Kinase: 0.4 µg/ml
• Filter binding assay
MSFC membrane (Millipore)

Additional assay technology:
IRAK4 Lot 006 was also successfully tested by Reaction Biology for the use with the ADP-Glo™ Kinase assay from Promega ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details

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ProQinase™ IRAK4 (untagged)

Product No.: 0586-0000-1

IRAK4 Recombinant Fusion Protein Amino Acid Sequence

1	GPLAM**AVPKT ANTLPSKEAI TVQQKQMPFC DKDRTLMTPV QNLEQSYMPP DSSSPENKSL**	60
61	**EVSDTRFHSF SFYELKNVTN NFDERPISVG GNKMGEGGFG VVYKGYVNNT TVAVKKLAAM**	120
121	**VDITTEELKQ QFDQEIKVMA KCQHENLVEL LGFSSDGDDL CLVYVYMPNG SLLDRLSCLD**	180
181	**GTPPLSWHMR CKIAQGAANG INFLHENHHI HRDIKSANIL LDEAFTAKIS DFGLARASEK**	240
241	**FAQTVMTSRI VGTTAYMAPE ALRGEITPKS DIYSFGVVLL EIITGLPAVD EHREPQLLLD**	300
301	**IKEEIEDEEK TIEDYIDKKM NDADSTSVEA MYSVASQCLH EKKNKRPDIK KVQQLLQEMT**	360
361	**AS**	420

1-5: remnant of tag cleavage **blue**: IRAK4 fragment

IRAK4 wt[1] Amino Acid Sequence

1	MNKPITPSTY VRCLNVGLIR KLSDFIDPQE GWKKLAVAIK KPSGDDRYNQ FHIRRFEALL	60
61	QTGKSPTSEL LFDWGTTNCT VGDLVDLLIQ NEFFAPASLL LPD**AVPKTAN TLPSKEAITV**	120
121	**QQKQMPFCDK DRTLMTPVQN LEQSYMPPDS SSPENKSLEV SDTRFHSFSF YELKNVTNNF**	180
181	**DERPISVGGN KMGEGGFGVV YKGYVNNTTV AVKKLAAMVD ITTEELKQQF DQEIKVMAKC**	240
241	**QHENLVELLG FSSDGDDLCL VYVYMPNGSL LDRLSCLDGT PPLSWHMRCK IAQGAANGIN**	300
301	**FLHENHHIHR DIKSANILLD EAFTAKISDF GLARASEKFA QTVMTSRIVG TTAYMAPEAL**	360
361	**RGEITPKSDI YSFGVVLLEI ITGLPAVDEH REPQLLLDIK EEIEDEEKTI EDYIDKKMND**	420
421	**ADSTSVEAMY SVASQCLHEK KNKRPDIKKV QQLLQEMTAS**	480

blue: IRAK4 sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_057207.2

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