Certificate of Analysis

ProQinase TARGETING CANCER

Recombinant Proteins

IRAK4 (GST-HIS-tag)

interleukin-1 receptor associated kinase 4

Recombinant Human Active Protein Kinase

HGNC Symbol: IRAK4

Synonyms: REN64; NY-REN-64; IPD1

Product No.: 0268-0000-1

Lot: 005

Description: Human IRAK4, C-terminal fragment, amino acids A_{104}-S_{460} (as in NCBI/Protein entry NP_057207.2), N-terminally fused to GST-HIS_6 and a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: IRAK4 Lot 005, was confirmed as IRAK4 by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 69,898 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

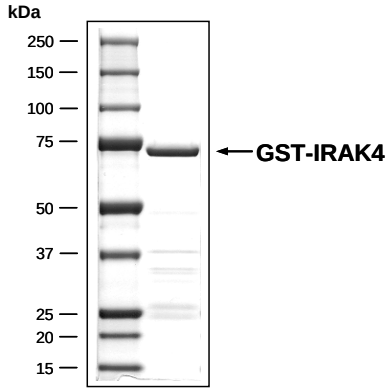
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 4 mM reduced glutathione, 20 % glycerol

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

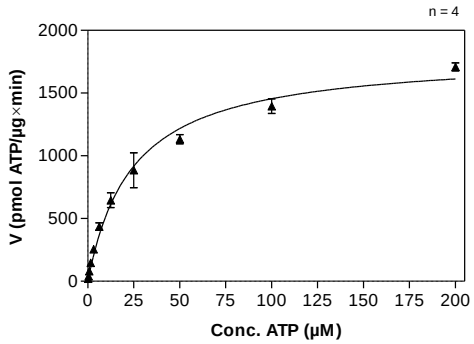
Biochemical Parameters:
Specific kinase activity (P_i transfer): 1809 pmol/µg × min
ATP-K_M: 24 µM

IRAK4 Lot 005:
Coomassie stain



2.0 µg GST-IRAK4

IRAK4 Lot 005:
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: Histon H2B 100 µg/ml
  Kinase: 0.4 µg/ml
- Filter binding assay

MSFC membrane (Millipore)

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IRAK4 (GST-HIS-tag)

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GST-IRAK4 Recombinant Fusion Protein Amino Acid Sequence

```
  1  MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID  60
 61  GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV 120
121  DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK 180
181  KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD PMGHHHHHHG RRRASVAAGI 240
241  LVPRGSPGLD GICSIEEFTM AVPKTANTLP SKEAITVQQK QMPFCDKDRT LMTPVQNLEQ 300
301  SYMPPDSSSP ENKSLEVSDT RFHSFSFYEL KNVTNNFDER PISVGGNKMG EGGFGVVYKG 360
361  YVNNTTVAVK KLAAMVDITT EELKQQFDQE IKVMAKCQHE NLVELLGFSS DGDDLCLVYV 420
421  YMPNGSLLDR LSCLDGTPPL SWHMRCKIAQ GAANGINFLH ENHHIHRDIK SANILLDEAF 480
481  TAKISDFGLA RASEKFAQTV MTSRIVGTTA YMAPEALRGE ITPKSDIYSF GVVLLEIITG 540
541  LPAVDEHREP QLLLDIKEEI EDEEKTIEDY IDKKMNDADS TSVEAMYSVA SQCLHEKKNK 600
601  RPDIKKVQQL LQEMTAS                                               660
```

1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: IRAK4 fragment

IRAK4 wt[1] Amino Acid Sequence

```
1    MNKPITPSTY VRCLNVGLIR KLSDFIDPQE GWKKLAVAIK KPSGDDRYNQ FHIRRFEALL  60
61   QTGKSPTSEL LFDWGTTNCT VGDLVDLLIQ NEFFAPASLL LPDAVPKTAN 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

Recombinant Proteins