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Certificate of Analysis

ICK, active
(Recombinant enzyme expressed in Sf21 insect cells)
Item # 15-023, 15-023-K, 15-023M
Parent Lot # 202962

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, amino acids 1-312 expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography.
Purity 69% by SDS-PAGE and Coomassie blue staining. MW = 40kDa.

Specific Activity (Parent lot# 202962): 520U/mg, where one unit of ICK activity is defined as 1nmol phosphate incorporated into 250µM RRRFRPASPLRGPPK per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.25mg/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.

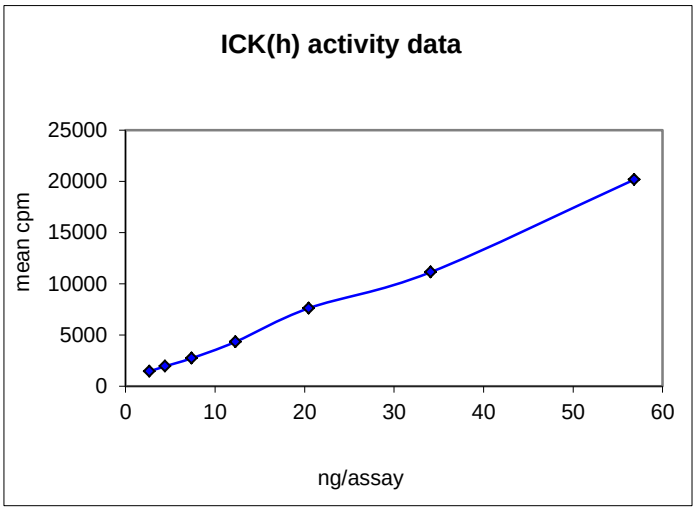
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

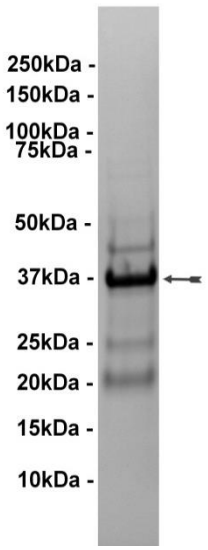
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 2.7–56.8ng of this lot of enzyme phosphorylated 250µM RRRFRPASPLRGPPK 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 ICK with the translated sequence listed on page three.



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

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. RRRFRPASPLRGPPK: Use at a final assay concentration of 250µM. Prepare a 2.5mM stock and add 2.5µl of stock per assay point.
3. **Enzyme:, active** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 2.7–56.8ng per assay point.
4. **[γ-^{33}P]ATP:** 2.5 x MgAc/[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 RRRFRPASPLRGPPK.
3. Add **2.5µl (2.7–56.8ng) ICK, 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 dried 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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ICK, active Sequence Information

Protein	Human
Tags	*N*-terminal 6His
Native sequence	M31 of the recombinant protein is equivalent to M1 of human ICK
Accession number	GenBank NM_014920.2

Recombinant ICK amino acid sequence:

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   1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF MNRYTTIRQL GDGTYGSVLL GRSIESGELI
  61 AIKKMKRKFY SWEECMNLRE VKSLKKLNHA NVVKLKEVIR ENDHLYFIFE YMKENLYQLI
 121 KERNKLFPES AIRNIMYQIL QGLAFIHKHG FFHRDLKPEN LLCMGPELVK IADFGLAREI
 181 RSKPPYTDYV STRWYRAPEV LLRSTNYSSP IDVWAVGCIM AEVYTLRPLF PGASEIDTIF
 241 KICQVLGTPK KTDWPEGYQL SSAMNFRWPQ CVPNNLKTLI PNASSEAVQL LRDMLQWDPK
 301 KRPTASQALR YPYFQVGHPL GSTTQNLQDS EKPQKGILEK AG
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Recombinant ICK nucleotide sequence:

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   1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
  61 tattttcagg gcgccatgga tccggaattc atgaatagat acacaacaat caggcagctc
 121 ggggatggaa cctacggttc cgtcctgctg ggaagaagca ttgagtctgg ggagctgatc
 181 gctattaaaa aaatgaaaag aaaattttat tcctgggagg aatgcatgaa ccttcgggag
 241 gttaagtctt taaagaagct caaccatgcc aatgtagtca aattaaaaga agttatcagg
 301 gaaaatgatc atctttattt tatcttcgag tacatgaagg aaaatcttta ccagctcatt
 361 aaagagagaa ataagttgtt tcctgagtct gctataagga atatcatgta tcagatatta
 421 caaggactcg catttattca caaacacggc ttctttcatc gagacttaaa gcctgagaac
 481 ctcctctgca tgggaccaga acttgtgaaa attgcagact ttggtttggc ccgagaaata
 541 cgatcaaaac ctccatatac agattatgta tctaccagat ggtacagggc tccagaagta
 601 ctcctgaggt ctaccaacta cagctccccc attgacgtct gggcggtggg ctgcatcatg
 661 gcagaagttt acaccctcag gccactcttc cctggagcca gtgaaattga cacaatattc
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 781 tcaagtgcaa tgaacttccg ttggccacag tgtgtaccca ataacttaaa gaccttgatt
 841 cccaatgcta gcagtgaagc agtccagctc ctgagagaca tgcttcagtg ggatcccaag
 901 aaacgaccaa cagctagtca ggcacttcga tatccttact tccaagttgg acacccacta
 961 ggcagcacca cacaaaacct tcaggattca gaaaaaccac agaaaggcat cctggaaaag
1021 gcaggctaa
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Reviewed and approved by site quality representative.