

Certificate of Analysis

IRE1, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-930, 14-930-K, 14-930M

Parent Lot # D13HP010N

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 IRE1 amino acids 465-end expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography. Purity 93.2% by SDS-PAGE and Coomassie blue staining. MW = 62kDa.

Specific Activity (Parent lot# D13HP010N): 393U/mg, where one unit of IRE1, active activity is defined as 1nmol phosphate incorporated into 0.33mg/ml MBP per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.478mg/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.

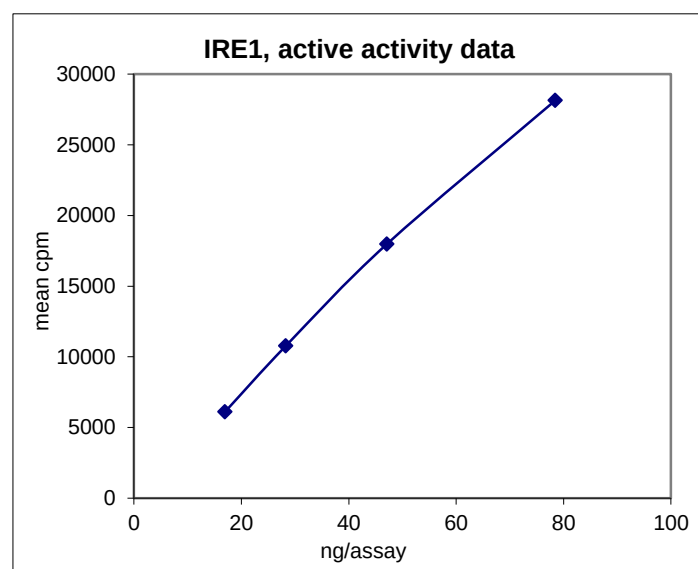
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

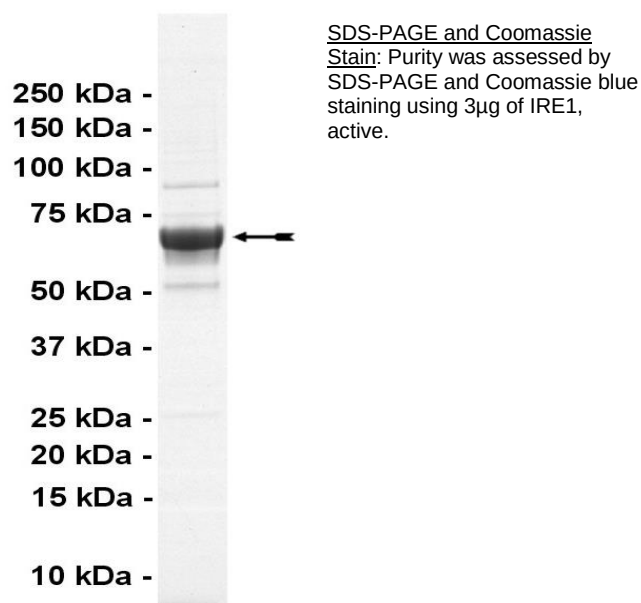
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 17–78ng of this lot of enzyme phosphorylated 0.33mg/ml 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 identity as IRE1 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. **MBP:** Use at a final assay concentration of 0.33mg/ml. Prepare a 3.33mg/ml stock and add 2.5µl of stock per assay point.
3. **10% BSA:** Use at a final assay concentration of 0.5%(w/v). Prepare a 10%(w/v) stock and add 1.25µl of stock per assay point
4. **IRE1, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 17–78ng per assay point.
5. **[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 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of MBP.
3. Add 1.25µl 10% BSA
4. Add **2.5µl (17–78ng) IRE1.**
5. Add 3.75µl of dH₂O.
6. Add 10µl of diluted [γ -³³P]ATP mixture.
7. Incubate for 10 minutes at 30°C.
8. Stop the reaction by adding 5µl of 3% phosphoric acid.
9. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat.**
10. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
11. Wash the filtermat once for 2 minutes with methanol.
12. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
13. 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.

IRE1 Sequence Information

Protein	human IRE1
Tags	N-terminal 6His
Native sequence	P31 of the recombinant protein is equivalent to P465 of human IRE1
Accession number	GenBank NM_001433

Recombinant IRE1 amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF PLSMHQQQQL QHQQFQKELE KIQLLQQQQQ
61 QLPFHPPGDT AQDGELLDTG GPYSESSGTS SPSTSPRASN HSLCSGSSAS KAGSSPSLEQ
121 DDGDEETSVV IVGKISFCPK DVLGHGAEGT IVYRGMFDNR DVAVKRILPE CFSFADREVQ
181 LLRESDEHPN VIRYFCTEKD RQFYIAIEL CAATLQEYVE QKDFAHGLLE PITLLQQTTS
241 GLAHLHSLNI VHRDLKPHNI LISMPNAHGK IKAMISDFGL CKKLAVGRHS FSRRSGVPGT
301 EGWIAPEMLS EDCKENPTYT VDI FSAGCVF YYVISEGSHF FGKSLQRQAN ILLGACSLDC
361 LHPEKHEDVI ARELIEKMIA MDPQKRPSAK HVLKHPFFWS LEKQLQFFQD VSDRIEKESL
421 DGPIVKQLER GGRAVVKMDW RENITVPLQT DLRKFRTYKG GSVRDLLRAM RNKKHHYREL
481 PAEVRETLGS LPDDFVCYFT SRFPHLLAHT YRAMELCSHE RLFQPYFHE PPEPQPPVTP
541 DAL

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Recombinant IRE1 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tatttttcagg gcgcattgga tccggaattc cccctgagca tgcattcagca gcagcagctc
121 cagcaccagc agttccagaa ggaactggag aagatccagc tcctgcagca gcagcagcag
181 cagctgccct tccaccacc tggagacacg gctcaggacg gcgagctcct ggacacgtct
241 ggcccgtact cagagagctc gggcaccagc agccccagca cgtccccccag ggcctccaac
301 cactcgtctc gctccggcag ctctgcctcc aaggctggca gcagccccctc cctggaacaa
361 gacgatggag atgaggaaac cagcgtgggtg atagtgggga aaatttcctt ctgtcccaag
421 gatgtcctgg gccattggagc tgagggcaca attgtgtacc ggggcatgtt tgacaaccgc
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1441 cctgcagagg tgcgggagac gctgggggtc ctccccgacg acttcgtgtg ctacttcacg
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1561 agactcttcc agccctacta cttccacgag ccccagagc cccagcccc agtgactcca
1621 gacgccctct ga

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