

## ERN1 (IRE1), Active

Recombinant human protein expressed in Sf9 cells

**Catalog # E31-11G**

Lot # G1464-1

### Product Description

Recombinant human ERN1 (IRE1) (468-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The ERN1 (IRE1) gene accession number is [NM\\_001433](#).

### Gene Aliases

hIRE1p; IRE1; IRE1A; IRE1P

### Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 25% glycerol.

### Storage and Stability

Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

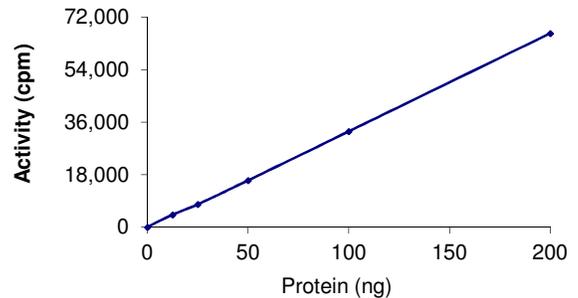
### Scientific Background

ERN1 or endoplasmic reticulum to nucleus signaling 1 is the ER to nucleus signalling 1 protein, a human homologue of the yeast Ire1 gene product which possesses intrinsic kinase activity and an endoribonuclease activity and it is important in endoplasmic reticulum-based stress signals. ERN1 controls IRE1 proteolysis in mammalian cells (1). The activation of ERN1 through oligomerization expands the mechanistic repertoire of kinase-based signaling receptors. ERN1 has a critical function in extraembryonic cells that is essential for fetal viability (2).

### References

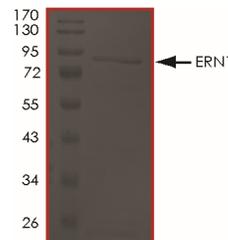
1. Niwa, M.et.al: A role for presenilin-1 in nuclear accumulation of Ire1 fragments and induction of the mammalian unfolded protein response. Cell 99: 691-702, 1999.
2. Iwawaki, T.et.al: Function of IRE1 alpha in the placenta is essential for placental development and embryonic viability. Proc. Nat. Acad. Sci. 106: 16657-16662, 2009.

### Specific Activity



The specific activity of ERN1 (IRE1) was determined to be **55 nmol /min/mg** as per activity assay protocol.

### Purity



The purity of ERN1 (IRE1) was determined to be **>90%** by densitometry. Approx. MW **87kDa**.

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Recombinant human protein expressed in Sf9 cells

<b>Catalog #</b>	<b>E31-11G</b>
<b>Specific Activity</b>	<b>55 nmol/min/mg</b>
<b>Lot #</b>	<b>G1464-1</b>
<b>Purity</b>	<b>&gt;90%</b>
<b>Concentration</b>	<b>0.1 µg/µl</b>
<b>Stability</b>	<b>1yr at -70°C from date of shipment</b>
<b>Storage &amp; Shipping</b>	<b>Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.</b>

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# Activity Assay Protocol

## Reaction Components

### Active Kinase (Catalog #: E31-11G)

Active ERN1 (IRE1) (0.1µg/µl) diluted with Kinase Dilution Buffer III (Catalog #: K23-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active ERN1 (IRE1) for optimal results).

### Kinase Dilution Buffer III (Catalog #: K23-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50 ng/µl BSA solution.

### Kinase Assay Buffer I (Catalog #: K01-09)

Buffer components: 25mM MOPS, pH 7.2, 12.5mM β-glycerol-phosphate, 25mM MgCl<sub>2</sub>, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

### [<sup>33</sup>P]-ATP Assay Cocktail

Prepare 250µM [<sup>33</sup>P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [<sup>33</sup>P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 1ml aliquots at -20°C.

### 10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 200µl aliquots at -20°C.

### Substrate (Catalog #: M42-51N)

Myelin basic protein (MBP) diluted in distilled H<sub>2</sub>O to a final concentration of 1mg/ml.

## Assay Protocol

- Step 1.** Thaw [<sup>33</sup>P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active ERN1 (IRE1), Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.
- Step 3.** In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20µl:
  - Component 1.** 10µl of diluted Active ERN1 (IRE1) (Catalog #E31-11G)
  - Component 2.** 5µl of 1mg/ml stock solution of substrate (Catalog #M42-51N)
  - Component 3.** 5µl distilled H<sub>2</sub>O (4°C)
- Step 4.** Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H<sub>2</sub>O.
- Step 5.** Initiate the reaction by the addition of 5µl [<sup>33</sup>P]-ATP Assay Cocktail bringing the final volume up to 25µl and incubate the mixture in a water bath at 30°C for 15 minutes.
- Step 6.** After the 15 minute incubation period, terminate the reaction by spotting 20µl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
- Step 7.** Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and ERN1 (IRE1) in a 1L solution with distilled H<sub>2</sub>O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
- Step 8.** Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
- Step 9.** Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

### Calculation of [<sup>33</sup>P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5µl [<sup>33</sup>P]-ATP / pmoles of ATP (in 5µl of a 250µM ATP stock solution, i.e., 1250 pmoles)

### Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)

Corrected cpm from reaction / [(SA of <sup>33</sup>P-ATP in cpm/pmol)\*(Reaction time in min)\*(Enzyme amount in µg or mg)]\*[(Reaction Volume) / (Spot Volume)]

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