

## EIF2AK1 (HRI), Active

Recombinant full-length protein expressed in Sf9 cells

Catalog # H07-10G

Lot # Y1056-2

### Product Description

Recombinant full-length human EIF2AK1 (HRI) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM\\_014413](#).

### Gene Aliases

HCR; HRI; KIAA1369

### 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.

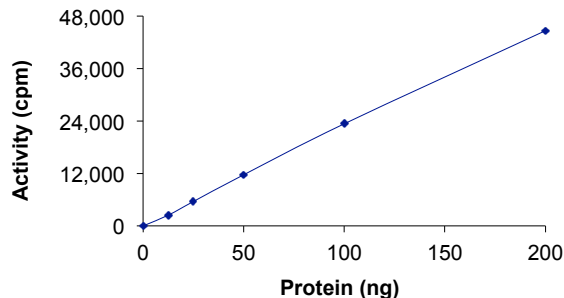
### Scientific Background

EIF2AK1 (HRI) or eukaryotic translation initiation factor 2-alpha kinase 1 acts at the level of translation initiation to downregulate protein synthesis in response to stress. EIF2AK1 (HRI) is a kinase that can be inactivated by hemin and is activated by heme deficiency and other stimuli and is a major protein kinase that phosphorylates EIF2-alpha (1). EIF2AK1 (HRI) is downregulated in the majority of ovarian cancers compared with normal ovarian tissues. EIF2AK1 (HRI) functions in iron homeostasis and may play a role in hemolytic and inflammatory anemia (2).

### References

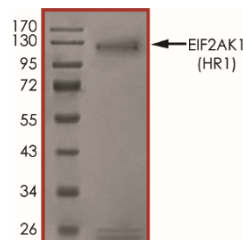
- Hwang, et.al: Cloning of hHRI, human heme-regulated eukaryotic initiation factor 2-alpha kinase: down-regulated in epithelial ovarian cancers. *Molec. Cells* 10: 584-591, 2000.
- Liu, S. et.al: The function of heme-regulated eIF2-alpha kinase in murine iron homeostasis and macrophage maturation. *J. Clin. Invest.* 117: 3296-3305, 2007.

### Specific Activity



The specific activity of EIF2AK1 (HRI) was determined to be **13 nmol/min/mg** as per activity assay protocol.

### Purity



The purity of EIF2AK1 (HRI) was determined to be **>70%** by densitometry, approx. MW **120 kDa**.

## EIF2AK1 (HRI), Active

Recombinant full-length human protein expressed in Sf9 cells

Catalog #	H07-10G
Specific Activity	13 nmol/min/mg
Lot #	Y1056-2
Purity	>70%
Concentration	0.05 µg/µl
Stability	1 yr at -70°C from date of shipment
Storage & Shipping	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.

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# Activity Assay Protocol

## Reaction Components

### Active Kinase (Catalog #: H07-10G)

Active EIF2AK1 (HRI) (0.05 $\mu$ g/ $\mu$ l) 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 EIF2AK1 (HRI) 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 50ng/ $\mu$ l BSA solution.

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

Buffer components: 25mM MOPS, pH 7. 2, 12.5mM  $\beta$ -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 250 $\mu$ M [<sup>33</sup>P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150 $\mu$ l of 10mM ATP Stock Solution (Catalog #: A50-09), 100 $\mu$ l [<sup>33</sup>P]-ATP (1mCi/100 $\mu$ l), 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 200 $\mu$ l aliquots at -20°C.

### Substrate (Catalog #: R55-58)

RS Repeat Peptide substrate (GRSRRSRSRSRSRSR) 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 EIF2AK1 (HRI), 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 20 $\mu$ l:
  - Component 1.** 10 $\mu$ l of diluted Active EIF2AK1 (HRI) (Catalog #H07-10G)
  - Component 2.** 5 $\mu$ l of 1mg/ml stock solution of substrate (Catalog #R55-58)
  - Component 3.** 5 $\mu$ l 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 5  $\mu$ l [<sup>33</sup>P]-ATP Assay Cocktail bringing the final volume up to 25 $\mu$ l 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 20  $\mu$ l 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 make 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 5  $\mu$ l [<sup>33</sup>P]-ATP / pmoles of ATP (in 5  $\mu$ l of a 250  $\mu$ M ATP stock solution, i.e., 1250 pmoles)

### Kinase Specific Activity (SA) (pmol/min/ $\mu$ g 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  $\mu$ g or mg)]\*[(Reaction Volume) / (Spot Volume)]

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