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1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
H07-10G -05	5 µg
H07-10G -10	10 µg

EIF2AK1 (HRI), Active

Recombinant full-length protein expressed in Sf9 cells

Catalog # H07-10G

Lot # Q2499-3

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.

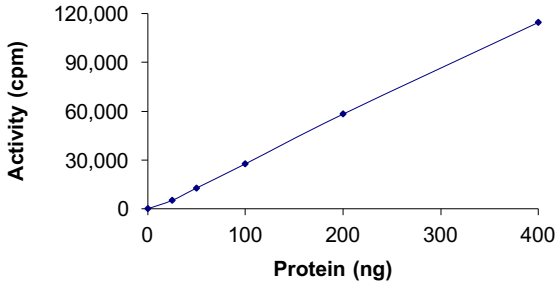
Scientific Background

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

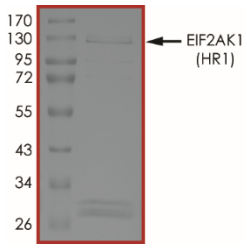
1. Hwang. et.al: Cloning of hHRI, human heme-regulated eukaryotic initiation factor 2-alpha kinase: down-regulated in epithelial ovarian cancers. Molec. Cells 10: 584-591, 2000.
2. Liu, S. et.al: The function of heme-regulated eIF2-alpha kinase in murine iron homeostasis and macrophage maturation. J.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

Specific Activity



The specific activity of EIF2AK1 (HRI) was determined to be **11 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	11 nmol/min/mg
Lot #	Q2499-3
Purity	>70%
Concentration	0.05 µg/µl
Stability	1yr 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) (0.05µg/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 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/µl BSA solution.

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

Buffer components: 25mM MOPS, pH 7. 2, 12.5mM β-glycerol-phosphate, 25mM $MgC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[^{33}P]-ATP Assay Cocktail

Prepare 250µM [^{33}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 [^{33}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 #: R55-58)

RS Repeat Peptide substrate (GRSRSRSRSRSRSRSR) diluted in distilled H_2O to a final concentration of 1mg/ml.

Assay Protocol

Step 1. Thaw [^{33}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 20µl:

- **Component 1.** 10µl of diluted Active EIF2AK1 (HRI) (Catalog #H07-10G)
- **Component 2.** 5µl of 1mg/ml stock solution of substrate (Catalog #R55-58)
- **Component 3.** 5µl distilled H_2O (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_2O.

Step 5. Initiate the reaction by the addition of 5 µl [^{33}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 20 µl 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_2O) 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 [P^{33}]-ATP Specific Activity (SA) (cpm/pmol)

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

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

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

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