EIF2AK4 (GCN2), Active
Recombinant human protein expressed in Sf9 cells
Catalog # E12-11G
Lot # L2124-5

Product Description
Recombinant human EIF2AK4 (192-1024) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_001013703.

Gene Aliases
GCN2; KIAA1338

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
EIF2AK4 or eukaryotic translation initiation factor 2 alpha kinase 4 belongs to a family of kinases that phosphorylate the alpha subunit of eukaryotic translation initiation factor-2 to downregulate protein synthesis in response to varied cellular stresses (1). The EIF2AK4 eIF2-alpha kinase regulates fatty-acid homeostasis in the liver during deprivation of an essential amino acid (2).

References

Specific Activity
The specific activity of EIF2AK4 was determined to be 16 nmol/min/mg as per activity assay protocol.

Purity
The purity of EIF2AK4 was determined to be >90% by densitometry, approx. MW 132kDa.

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Activity Assay Protocol

Reaction Components

**Active Kinase** (Catalog #: E12-11G)

Active EIF2AK4 (0.1µg/µl) diluted with Kinase Dilution Buffer IV (Catalog #: K24-09) 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 EIF2AK4 for optimal results).

**Kinase Dilution Buffer IV** (Catalog #: K24-09)

Kinase Assay Buffer II (Catalog #: K02-09) diluted at a 1:4 ratio (5X dilution) with 50ng/µl BSA solution.

**Kinase Assay Buffer II** (Catalog #: K02-09)

Buffer components: 25mM MOPS, pH 7.2, 12.5mM β-glycerol-phosphate, 20mM MgCl₂, 12.5mM MnCl₂, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

**[³³P]-ATP Assay Cocktail**

Prepare 250µM [³³P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [³³P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer II (Catalog #: K02-09). 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 distilled H₂O to a final concentration of 1mg/ml.

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

RS Repeat Peptide (GRSRSRSRSRSRSRSR) diluted in distilled H₂O to a final concentration of 1mg/ml.

**Assay Protocol**

1. Thaw [³³P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
2. Thaw the Active EIF2AK4, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.
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 EIF2AK4 (Catalog #E12-11G)
   Component 2. 5µl of 1mg/ml stock solution of substrate (Catalog #R55-58)
   Component 3. 5µl of distilled H₂O
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 H₂O.
5. Initiate the reaction by the addition of 5 µl [³³P]-ATP Assay Cocktail bringing the final volume up to 25µl and incubate the mixture in a water bath at 30°C for 15 minutes.
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.
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 H₂O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
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 [P³³]-ATP Specific Activity (SA) (cpm/pmol)**

Specific activity (SA) = cpm for 5 µl [³³P]-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 [³³P]-ATP in cpm/pmol) * (Reaction time in min) * (Enzyme amount in µg or mg)] / [(Reaction Volume) / (Spot Volume)]

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SAFETY DATA SHEET

Article 1 - Product Identification

Product Name: EIF2AK4 (GCN2), Active
Catalog # E12-11G
This product is sold only for research use by qualified laboratory personnel, and is not to be used as a drug, medical device, food additive, cosmetic, nor household chemical. It is not to be used in diagnostic, therapeutic, consumer, agricultural, nor pesticidal applications.

Manufacturer’s Name: SignalChem Biotech Inc.
Street Address: 110-13120 Vanier Place
City, Prov. Postal Code: Richmond, BC, V6V 2J2
Fax: 604-232-4601
EMERGENCY PHONE: 604-232-4600

Article 2 - Hazard Identification

- WHMIS Classification: Not WHMIS controlled.
- GHS classification: Skin irritation (Category 3); Eye irritation (Category 2B).
- Hazard Pictograms: none.
- Signal words: Warning.
- Hazard statements: Causes mild skin irritation (H316); Causes eye irritation (H320).
- Precautionary statements: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. (P305 + P351 + P338).
- Other hazards: none known.

Article 3 - Composition/Information on Ingredients

Chemical Characterization: Mixtures.
Description: This product consists of the substances listed below.

<table>
<thead>
<tr>
<th>Common name</th>
<th>Chemical name</th>
<th>CAS-No.</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol</td>
<td>Glycerol</td>
<td>56-81-5</td>
<td>≤25%</td>
</tr>
<tr>
<td>NaCl</td>
<td>Sodium chloride</td>
<td>7647-14-5</td>
<td>≤1.753 %</td>
</tr>
<tr>
<td>Tris-HCl; Tris (hydroxymethyl) aminomethane hydrochloride</td>
<td>2 – Amino – 2 - (hydroxymethyl) propane - 1, 3 - diol hydrochloride</td>
<td>1185-53-1</td>
<td>&lt;0.8%</td>
</tr>
<tr>
<td>Glutathione</td>
<td>Glutathione</td>
<td>70-18-8</td>
<td>0.307%</td>
</tr>
<tr>
<td>Protein</td>
<td>No data available</td>
<td></td>
<td>≤0.02%</td>
</tr>
<tr>
<td>DTT; Dithiothreitol</td>
<td>(R*,R*)-1,4-Dimercaptobutane-2,3-diol</td>
<td>3483-12-3</td>
<td>0.0038%</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
<td>6381-92-6</td>
<td>0.0037%</td>
</tr>
<tr>
<td>PMSF; Phenylmethanesulfonyl fluoride</td>
<td>o-Toluenesulphonyl fluoride</td>
<td>329-98-6</td>
<td>0.002%</td>
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</tbody>
</table>

Article 4 - First-aid Measures

- General information: Consult a physician by providing the SDS.
- After inhalation: Breathe in fresh air. If cannot breathe, give artificial respiration and consult a physician.
- After skin contact: Immediately wash with soap and plenty of water and rinse thoroughly. Consult a physician.
- After eye contact: Rinse opened eyes with plenty of water for at least 15 minutes. Consult a physician.
- After swallowing: rinse the mouth with plenty of water and consult a physician.

Article 5 - Fire-fighting Measures

- Suitable extinguishing media: Use water spray, extinguishing powder, carbon dioxide, or other appropriate measure that is suitable to the environment.
- Specific hazards arising from the substance or mixture: None known.
- Special protective equipment and precautions for fire-fighters: Self-contained breathing apparatus if necessary.

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Article 6 – Accidental Release Measures

- Personal precautions, protective equipment and emergency procedures: Apply standard laboratory practices and personal protective equipment. Avoid breathing vapors, mist, or gas. Ensure adequate ventilation.
- Environmental precautions: Do not allow to enter drains.
- Methods and materials for containment and cleaning up: Absorb on sand or vermiculite and place in closed containers for disposal.

Article 7 - Handling and Storage

- Precautions for safe handling: Wear chemical safety goggles and compatible chemical-resistant gloves. Avoid inhalation, contact with eyes, skin or clothing.
- Conditions for safe storage: Store in a dry and well-ventilated place in -70 °C. Keep container upright and tightly closed.

Article 8 - Exposure Controls/Personal Protection

- Components with limit monitoring values at workplace:
  Glycerol (CAS-No: 56-81-5)

<table>
<thead>
<tr>
<th>Values</th>
<th>Control parameters</th>
<th>Regulations</th>
</tr>
</thead>
<tbody>
<tr>
<td>TWA</td>
<td>10 mg/m³ for mist</td>
<td>British Columbia, Canada</td>
</tr>
<tr>
<td>TWA</td>
<td>3 mg/m³ for respirable</td>
<td>British Columbia, Canada</td>
</tr>
<tr>
<td>TWA</td>
<td>10 mg/m³</td>
<td>Alberta, Canada</td>
</tr>
<tr>
<td>TWA</td>
<td>10 mg/m³</td>
<td>Ontario, Canada</td>
</tr>
<tr>
<td>TWA</td>
<td>10 mg/m³</td>
<td>Quebec, Canada</td>
</tr>
<tr>
<td>TWA</td>
<td>10 mg/m³</td>
<td>USA</td>
</tr>
</tbody>
</table>

- Appropriate engineering controls:
  Apply adequate ventilation including mechanical exhaust or laboratory fume hood. Follow standard laboratory practices.
- Individual protection measures:
  Respiratory protection:
    Use appropriate respirator if there is inadequate ventilation by following the government standards.
  Hand protection:
    Wear gloves and use proper glove removal technique to avoid skin contact. Discard gloves after use by following the applicable laboratory regulations. Wash and dry hands.
  Eye/face protection:
    Safety goggles with side-shields approved under appropriate government standards.
  Skin/body protection:
    Use appropriate clothing, footwear and any additional protection measures to protect from splashing or contamination.

Article 9 – Physical and Chemical Properties

- Appearance: Colorless fluid.
- Odour/odour threshold: Not determined.
- pH: Not available.
- Melting point/freezing point: Not determined.
- Boiling point/boiling range: 100 °C.
- Flash point: > 100 °C.
- Flammability (solid, gaseous): Not determined.
- Ignition temperature: 400 °C.
- Auto-igniting: Product is not self-igniting.
- Danger of explosion: Product does not present an explosion hazard.
- Explosion limits: Lower: 0.9 Vol %; Upper: 0.0 Vol %.
- Decomposition temperature: Not available.
- Vapor pressure at 20 °C: 0.1 hPa.
- Relative density: Not determined.
- Vapor density: Not determined.
- Evaporation rate: Not determined.
- Solubility in / Miscibility with water: Fully miscible.

Article 10 - Stability and Reactivity

- Reactivity: Stable under recommended transport and storage conditions.
- Chemical stability: Stable under recommended transport and storage conditions.
- Possible hazardous reactions: No dangerous reactions known.
- Conditions to avoid: Heat and moisture.
- Incompatible materials: Strong acids/bases, strong oxidizing/reducing agents.
- Hazardous decomposition products: Carbon oxides may formed under fire conditions; no known decomposition information for other decomposition products.

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Article 11 - Toxicological Information

- Acute toxicity: Not available.
- LD/LC50: Not available.
- Skin corrosion/irritation: Not available.
- Serious eye damage/eye irritation: Not available.
- Respiratory or skin sensitization: Not available.
- Germ cell mutagenicity: Not available.
- Carcinogenicity: No components are listed in IARC, or NTP, or OSHA, or ACGIH.
- Reproductive toxicity: Not available.
- Teratogenicity: Not available.
- Specific target organ toxicity - single exposure/ - repeated exposure (GHS): Not available.
- Aspiration hazard: Not available.
- Potential health effects:
  - Inhalation: May be harmful if inhaled. May cause respiratory tract irritation.
  - Ingestion: May be harmful if swallowed.
  - Skin: May be harmful if absorbed through skin. May cause skin irritation.
  - Eyes: May cause eye irritation.
- Signs and Symptoms of Exposure:
  - Prolonged or repeated exposure can cause: Nausea, Dizziness.
- Synergistic effects: Not available.

Article 12 - Ecological Information

- Eco-toxicity: Not applicable.
- Biodegradability: Not applicable.
- Bio-accumulative potential: Not applicable.
- Mobility in soil: Not applicable.
- PBT and vPvB assessment: Not applicable.
- Other adverse effects: Not applicable.

Article 13 - Disposal Considerations

- Disposal methods: In accordance to applicable national, regional, or local laws and regulations. For additional handling information and protection of employees please refer to Article 7 and 8.
- Contaminated packaging: Disposal should be made in accordance to official regulations. Use water or cleansing agents to clean the area.

Article 14 - Transport Information

- DOT: Not dangerous goods.
- IMDG: Not dangerous goods.
- IATA: Not dangerous goods.

Article 15 – Regulatory Information

- WHMIS Classification: Non-hazardous.
- GHS label elements: Not applicable.
- Signal word: Not applicable.
- Hazard statements: Not applicable.

Article 16 - Other Information

The above information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide. SignalChem shall not be held liable for any damage resulting from handling or from contact with the above product. See the Technical Specification, Packing Slip, Invoice, and Product Catalog for additional terms and conditions of sale.

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