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Product Information

EIF2AK3 (563-1115), active, GST-tagged, human
PRECISIO® Kinase
recombinant, expressed in *E. coli* cells

Catalog Number **SRP5024**
Storage Temperature –70 °C

Synonyms: PERK, PEK, WRS, HRI, DKFZp781H1925

Product Description

EIF2AK3 phosphorylates the alpha subunit of eukaryotic translation-initiation factor 2 (EIF2) leading to its inactivation, and a rapid reduction of translational initiation and repression of global protein synthesis. EIF2AK3 is a type I membrane protein located in the endoplasmic reticulum (ER), where it is induced by ER stress caused by malfolded proteins.[1] EIF2AK3 plays a major role in the ability of cells to adapt to ER stress and is also involved in an integrated adaptive response to hypoxic stress in HeLa cells.[2] EIF2AK3 functions in iron homeostasis and may play a role in hemolytic and inflammatory anemia.

Recombinant human EIF2AK3 (563-1115) was expressed in *E. coli* cells using an N-terminal GST tag. The gene accession number is NM_004836. Recombinant protein stored in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM glutathione, 0.1 mM EDTA, 0.25 mM DTT, 0.1 mM PMSF, and 25% glycerol.

Molecular mass: ~115 kDa

Purity: 70–95% (SDS-PAGE, see Figure 1)

Specific Activity: 15–21 nmole/min/mg (see Figure 2)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The product ships on dry ice and storage at –70 °C is recommended. After opening, aliquot into smaller quantities and store at –70 °C. Avoid repeated handling and multiple freeze/thaw cycles.

Figure 1.
SDS-PAGE Gel of Typical Lot
70–95% (densitometry)

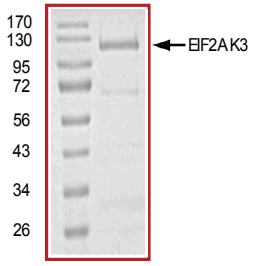
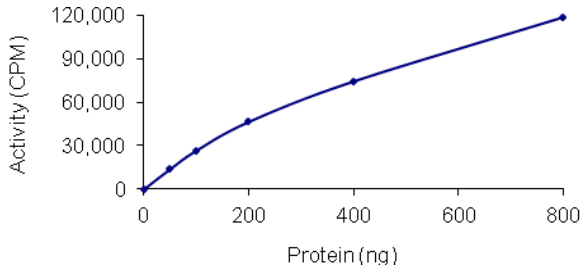


Figure 2.
Specific Activity of Typical Lot
15–21 nmole/min/mg



Procedure

Preparation Instructions

Kinase Assay Buffer – 25 mM MOPS, pH 7.2, 12.5 mM glycerol 2-phosphate, 20 mM $MgCl_2$, 5 mM EGTA, and 2 mM EDTA. Just prior to use, add DTT to a final concentration of 0.25 mM.

Kinase Dilution Buffer – Dilute the Kinase Assay Buffer 5-fold with a 50 ng/µl BSA solution.

Kinase Solution – Dilute the active EIF2AK3 (563-1115) (0.1 μg/μl) with Kinase Dilution Buffer to the desired concentration.
Note: The lot-specific specific activity plot may be used as a guideline (see Figure 2). It is recommended the researcher perform a serial dilution of active EIF2AK3 (563-1115) kinase for optimal results.

10 mM ATP Stock Solution – Dissolve 55 mg of ATP in 10 ml of Kinase Assay Buffer. Store in 200 μl aliquots at –20 °C.

γ-^{33}P-ATP Assay Cocktail (250 μM) – Combine 5.75 ml of Kinase Assay Buffer, 150 μl of 10 mM ATP Stock Solution, 100 μl of γ-^{33}P-ATP (1 mCi/100 μl). Store in 1 ml aliquots at –20 °C.

Substrate Solution – SMAD3 protein at concentration of 0.2 μg/μl.

1% phosphoric acid solution – Dilute 10 ml of concentrated phosphoric acid to a final volume of 1 L with water.

Kinase Assay
This assay involves the use of the ^{33}P radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Thaw the active EIF2AK3 (563-1115), Kinase Assay Buffer, Substrate Solution, and Kinase Dilution Buffer on ice. The γ-^{33}P-ATP Assay Cocktail may be thawed at room temperature.
2. In a pre-cooled microcentrifuge tube, add the following solutions to a volume of 20 μl:
   10 μl of Kinase Solution
   10 μl of Substrate Solution
3. Set up a blank control as outlined in step 2, substituting 10 μl of cold water (4 °C) for the Substrate Solution.
4. Initiate each reaction with the addition of 5 μl of the γ-^{33}P-ATP Assay Cocktail, bringing the final reaction volume to 25 μl. Incubate the mixture in a water bath at 30 °C for 15 minutes.
5. After the 15 minute incubation, stop the reaction by spotting 20 μl of the reaction mixture onto an individually precut strip of phosphocellulose P81 paper.
6. Air dry the precut P81 strip and sequentially wash in the 1% phosphoric acid solution with constant gentle stirring. It is recommended the strips be washed a total of 3 times of ~10 minutes each.
7. Set up a radioactive control to measure the total γ-^{33}P-ATP counts introduced into the reaction. Spot 5 μl of the γ-^{33}P-ATP Assay Cocktail on a precut P81 strip. Dry the sample for 2 minutes and read the counts. Do not wash this sample.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
9. Determine the corrected cpm by subtracting the blank control value (see step 3) from each sample and calculate the kinase specific activity

Calculations:
1. Specific Radioactivity (SR) of ATP (cpm/nmole)

$$SR = \frac{\text{cpm of 5 μl of γ-}^{33}\text{P-ATP Assay Cocktail}}{\text{nmole of ATP}}$$

   cpm – value from control (step 7)
   nmole – 1.25 nmole (5 μl of 250 μM ATP Assay Cocktail)

2. Specific Kinase Activity (SA) (nmole/min/mg)

$$\text{nmole/min/mg} = \frac{\Delta\text{cpm} \times (25/20)}{SR \times E \times T}$$

SR = specific radioactivity of the ATP (cpm/nmole ATP)
Δcpm = cpm of the sample – cpm of the blank (step 3)
25 = total reaction volume
20 = spot volume
T = reaction time (minutes)
E = amount of enzyme (mg)

References

1. Harding, H.P. et.al., EIF2AK3 is essential for translational regulation and cell survival during the unfolded protein response. Molec. Cell, **5**, 897-904 (2000).
2. Blais, J.D. et.al., Activating transcription factor 4 is translationally regulated by hypoxic stress. Molec. Cell. Biol., **24**, 7469-7482 (2004).

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