Product Information

ALK2 (147-end), active, GST-tagged, human
PRECISIO® Kinase recombinant, expressed in Sf9 cells

Catalog Number A4361
Storage Temperature –70 °C

Synonyms: ACVR1, ACTRI, ACVRL2, FOP, SKR1, TSRI

Product Description
ALK2 is a receptor serine/threonine kinase that is a member of the ALK family and is upstream of a signaling pathway involving the SMAD proteins especially SMAD1/5/8. Knockdown of ALK2, but not TGFβRI (ALK5), abrogates endoglin-mediated decrease in cell motility of prostate cancer cells. Constitutively active ALK2 is sufficient to restore a low-motility phenotype in endoglin deficient cells. Therefore, endoglin decreases prostate cancer cell motility through activation of the ALK2-Smad1 pathway. ALK2 is the key gene involved in fibrodysplasia ossificans progressiva (FOP), a rare autosomal dominant congenital disorder characterized by progressive heterotopic bone formation in muscle tissues.

This recombinant product was expressed by baculovirus in Sf9 insect cells using an N-terminal GST-tag. The gene accession number is NM 001105. It is supplied in 50 mM Tris-HCl, pH 7.5, with 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, and 25% glycerol.

Molecular mass: ~67 kDa

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the 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% (SDS-PAGE, densitometry)

Figure 2.
Specific Activity of Typical Lot:
39–53 nmole/min/mg

Procedure
Preparation Instructions
Kinase Assay Buffer – 25 mM MOPS, pH 7.2, 12.5 mM glycerol 2-phosphate, 25 mM MgCl2, 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 ALK2 (0.1 µg/µl) with Kinase Dilution Buffer to the desired concentration. Note: The specific activity plot may be used as a guideline (see Figure 2). It is recommended that the researcher perform a serial dilution of active ALK2 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-32P-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-32P-ATP (1 mCi/100 µl). Store in 1 ml aliquots at −20 °C.

Substrate Solution – Dilute casein protein in water at a final concentration of 1 mg/ml.

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 32P radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Thaw the active ALK2, Kinase Assay Buffer, Substrate Solution, and Kinase Dilution Buffer on ice. The γ-32P-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
   - 5 µl of Substrate Solution
   - 5 µl of cold water (4 °C)
3. Set up a blank control as outlined in step 2, substituting 5 µl of cold water (4 °C) for the Substrate Solution.
4. Initiate each reaction with the addition of 5 µl of the γ-32P-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-32P-ATP counts introduced into the reaction. Spot 5 µl of the γ-32P-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)

\[ SR = \frac{\text{cpm of } 5 \mu l \text{ of } \gamma^{32p} \text{-ATP Assay Cocktail}}{\text{nmole of ATP}} \]

\[ \text{cpm} = \text{value from control (step 7)} - \text{nmole} \times 1.25 \text{ nmole (5 } \mu l \text{ of 250 } \mu M \text{ ATP Assay Cocktail)} \]

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

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

\[ \Delta \text{cpm} = \text{cpm of the sample} - \text{cpm of the blank (step 3)} \]

25 = total reaction volume

20 = spot volume

T = reaction time (minutes)

E = amount of enzyme (mg)

References

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