

ALK2 (R258G), Active

Recombinant protein expressed in Sf9 cells

Catalog # **A06-12KG**

Lot # D2374-10

Product Description

Recombinant human ALK2 (R258G) (147-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The ALK2 gene accession number is [NM_001105](#).

Gene Aliases

ACVR1, FOP, SKR1, TSRI, ACTRI, ACVR1A, ACVRLK2

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.

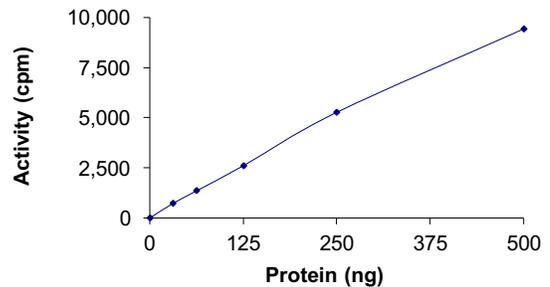
Scientific Background

ALK 2 is a receptor serine/threonine kinase that is member of the ALK family and is upstream of signaling pathway involving the SMAD proteins especially SMAD1/5/8. Knockdown of ALK2, but not TGFBR1 (ALK5), abrogates endoglin-mediated decrease in cell motility of prostate cancer cells and constitutively active ALK2 is sufficient to restore a low-motility phenotype in endoglin deficient cells (1). 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 (2).

References

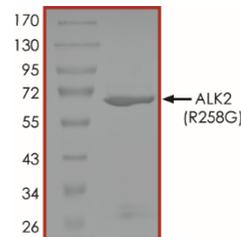
1. Craft, C.S. et al: Endoglin inhibits prostate cancer motility via activation of the ALK2-Smad1 pathway. *Oncogene*. 2007 Nov 8;26(51):7240-50.
2. Shore, E. M. et al: A recurrent mutation in the BMP type I receptor ACVR1 causes inherited and sporadic fibrodysplasia ossificans progressiva. *Nature Genet*. 38: 525-527, 2006.

Specific Activity



The specific activity of ALK2 (R258G) was determined to be **1.3 nmol /min/mg** as per activity assay protocol.

Purity



The purity of ALK2 (R258G) was determined to be **>95%** by densitometry, approx. MW **67 kDa**.

ALK2 (R258G), Active

Recombinant human protein expressed in Sf9 cells

Catalog #	A06-12KG
Specific Activity	1.3 nmol/min/mg
Lot #	D2374-10
Purity	>95%
Concentration	0.1 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 #: A06-12KG)

Active ALK2 (R258G) (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 ALK2 (R258G) 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 final 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, 5mM EGTA, 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 I (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 Kinase Assay Buffer I (Catalog #: K02-09). Store 200µl aliquots at -20°C.

Substrate (Catalog #: C03-54N)

Casein Protein substrate diluted in distilled H₂O to a final concentration of 1mg/ml.

Assay Protocol

- Step 1.** Thaw [³³P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active ALK2 (R258G), 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 ALK2 (R258G) (Catalog #A06-12KG)
 - Component 2.** 5µl of 1mg/ml stock solution of substrate (Catalog #C03-54N)
 - Component 3.** 5µl distilled H₂O (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 H₂O.
- Step 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.
- 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 H₂O) 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 [³³P]-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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