

## ALK4, Active

Recombinant human protein expressed in Sf9 cells

Catalog # A07-11G-10

Lot # C246-1

### Product Description

Recombinant human ALK4 (150-end) was expressed in Sf9 cells using a N-terminal GST tag. The gene accession number is [NM\\_004302](#).

### Gene Aliases

ACVR1B, SKR2, ACTRIB, ACVRLK4

### Concentration

0.1 µg/µl

### 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, Shipping 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.

Stability is 1yr at -70°C from date of shipment. Product shipped on dry ice.

### Scientific Background

ALK4 is a member of the subfamily of receptor ser/thr kinases that mediates signaling by the Activins. ALK4 is expressed in many human tissues, including kidney, pancreas, brain, lung, and liver. Truncated ALK4, predominantly expressed in human pituitary adenomas, function as dominant negative receptors to interfere with wild-type receptor function and blocks the antiproliferative effect of activin possibly contributing to development of human pituitary tumors (1). ALK4 is able to mediate Nodal signaling in the presence of Cripto during vertebrate development (2).

### References

1. Zhou, Y. et al: Truncated activin type I receptor Alk4 isoforms are dominant negative receptors inhibiting activin signaling. *Mol Endocrinol.* 2000 Dec;14(12):2066-75.
2. Reissmann, E. et al: The orphan receptor ALK7 and the Activin receptor ALK4 mediate signaling by Nodal proteins during vertebrate development. *Genes Dev.* 2001 Aug 1;15(15):2010-22.

### Purity

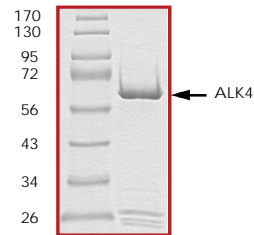
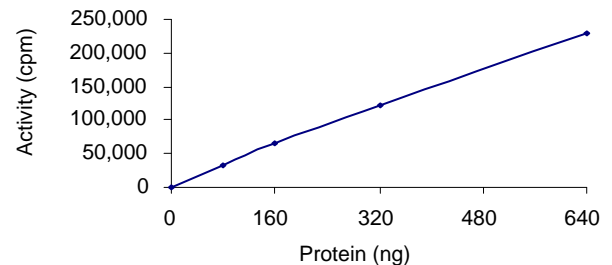


Figure 1. SDS-PAGE gel image

The purity of ALK4 was determined to be **>95%** by densitometry, approx. MW **~64kDa**.

### Specific Activity

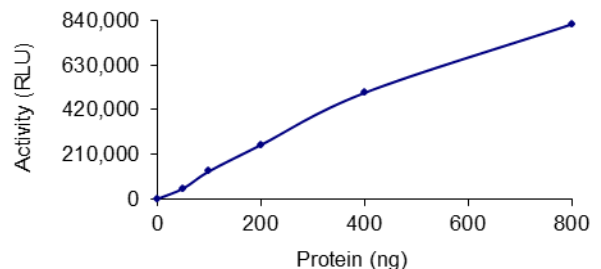
Figure 2. Radiometric Assay Data



The specific activity of ALK4 was determined to be **16 nmol /min/mg** as per activity assay protocol.

(For Radiometric Assay Protocol on this product please see pg. 2)

Figure 3. ADP-Glo™ Assay Data



The specific activity of ALK4 was determined to be **22 nmol /min/mg** as per activity assay protocol.

(For ADP-Glo™ Assay Protocol on this product please see pg. 3)

# Activity Assay Protocol

## Reaction Components

### Active Kinase (Catalog #: A07-11G)

Active ALK4 (0.1µg/µl) diluted with Kinase Dilution Buffer VII (Catalog #: K27-09) 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 ALK4 for optimal results).

### Kinase Dilution Buffer VII (Catalog #: K27-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 5% glycerol 50ng/µl BSA solution.

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

Buffer components: 25mM MOPS, pH 7.2, 12.5mM β-glycerol-phosphate, 25mM MgCl<sub>2</sub>, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

### [<sup>33</sup>P]-ATP Assay Cocktail

Prepare 250µM [<sup>33</sup>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 [<sup>33</sup>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 #: C03-54N)

Casein protein substrate, was diluted in distilled H<sub>2</sub>O to a final concentration of 1mg/ml.

## Assay Protocol

- Step 1. Thaw [<sup>33</sup>P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2. Thaw the Active ALK4, 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 ALK4 (Catalog #A07-11G)
  - Component 2. 5µl of 1mg/ml stock solution of substrate (Catalog #C03-54N)
  - Component 3. 5µl distilled H<sub>2</sub>O (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<sub>2</sub>O.
- Step 5. Initiate the reaction by the addition of 5µl [<sup>33</sup>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 20µl 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<sub>2</sub>O) 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 [<sup>33</sup>P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5µl [<sup>33</sup>P]-ATP / pmoles of ATP (in 5µl of a 250µM ATP stock solution, i.e., 1,250 pmoles)

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

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

# ADP-Glo™ Activity Assay Protocol

## Reaction Components

### ALK4 Kinase Enzyme System (Promega, Catalog #:V4508)

ALK4, Active, 10µg (0.1µg/µl)  
Casein Protein, 1ml (1mg/ml)  
Reaction Buffer A (5X), 1.5ml  
DTT solution (0.1M), 25µl

### ADP-Glo™ Kinase Assay Kit (Promega, Catalog #: V9101)

Ultra Pure ATP, 10 mM (0.5ml)  
ADP, 10 mM (0.5ml)  
ADP-Glo™ Reagent (5ml)  
Kinase Detection Buffer (10ml)  
Kinase Detection Substrate (Lyophilized)

### Reaction Buffer A (5X)

200mM Tris-HCl, pH 7.5, 100mM MgCl<sub>2</sub> and 0.5 µg/µl BSA.

## Assay Protocol

The ALK4 assay is performed using the ALK4 Kinase Enzyme System (Promega; Catalog #: V4508) and ADP-Glo™ Kinase Assay kit (Promega; Catalog #: V9101). The ALK4 reaction utilizes ATP and generates ADP. Then the ADP-Glo™ Reagent is added to simultaneously terminate the kinase reaction and deplete the remaining ATP. Finally, the Kinase Detection Reagent is added to convert ADP to ATP and the newly synthesized ATP is converted to light using the luciferase/luciferin reaction. For more detailed protocol regarding *the ADP-Glo™ Kinase Assay*, see the Technical Manual #TM313, available at [www.promega.com/tbs/tm313/tm313.html](http://www.promega.com/tbs/tm313/tm313.html).

- Step 1. Thaw the ADP-Glo™ Reagents at ambient temperature. Then prepare Kinase Detection Reagent by mixing Kinase Detection Buffer with the Lyophilized Kinase Detection Substrate. Set aside.
- Step 2. Thaw the components of ALK4 Enzyme System, ADP and ATP on ice.
- Step 3. Prepare 1ml of 2X Buffer by combining 400µl Reaction Buffer A, 1µl DTT and 599µl of dH<sub>2</sub>O.
- Step 4. Prepare 1ml of 250µM ATP Assay Solution by adding 25µl ATP solution (10mM) to 500µl of 2X Buffer and 475µl of dH<sub>2</sub>O.
- Step 5. Prepare diluted ALK4 in 1X Buffer (diluted from 2X buffer) 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 ALK4 for optimal results).
- Step 6. In a white 96-well plate (Corning Cat # 3912), add the following reaction components bringing the initial reaction volume up to 20µl:

Component 1.	10µl of diluted Active ALK4
Component 2.	5µl of 1mg/ml stock solution of substrate
Component 3.	5µl of 2X Buffer
- Step 7. Set up the blank control as outlined in step 6, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H<sub>2</sub>O.
- Step 8. At the same time as the ALK4 kinase reaction, set up an ATP to ADP conversion curve at 50µM ATP/ADP range as described in the *ADP-Glo™ Kinase Assay* Technical Manual #TM313.
- Step 9. Initiate the ALK4 reactions by the addition of 5µl of 250µM ATP Assay Solution thereby bringing the final volume up to 25µl. Shake the plate and incubate the reaction mixture at 30°C for 15 minutes.
- Step 10. Terminate the reaction and deplete the remaining ATP by adding 25µl of ADP-Glo™ Reagent. Shake the 96-well plate and then incubate the reaction mixture for another 40 minute at ambient temperature.
- Step 11. Add 50µl of the Kinase Detection Reagent, shake the plate and then incubate the reaction mixture for another 30 minute at ambient temperature.
- Step 12. Read the 96-well reaction plate using the Kinase-Glo™ Luminescence Protocol on a GloMax® Microplate Luminometer (Promega; Cat # E6501).
- Step 13. Using the conversion curve, determine the amount of ADP produced (nmol) in the presence (step 6) and absence of substrate (Step 7) and calculate the kinase specific activity as outlined below. For a detailed protocol of how to determine nmols from RLUs, see ADP-Glo™ Applications Database at <http://www.promega.com/applications/cellularanalysis/cellsignaling.htm>

### Kinase Specific Activity (SA) (nmol/min/mg)

(ADP (step 6) – ADP (Step 7)) in nmol) / (Reaction time in min)\*(Enzyme amount in mg)