

Recombinant Human Active Akt1

Certificate of Analysis

Catalog Number: 1775-KS
Lot Number: 1495375

Specifications and Use

- Source** ♦ Recombinant full-length human Akt1 was expressed by baculovirus in *Sf9* insect cells using an N-terminal GST tag. The gene accession number is NM_005163
- Molecular Mass** ♦ The approximate molecular weight is 85 kDa (see Figure 1 below).
- Purity** ♦ The purity was determined to be > 95% by densitometry (see Figure 1 below).
- Formulation** ♦ Supplied in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.25 mM DTT, 10 mM Glutathione, 0.1 mM EDTA, 0.1 mM PMSF, 25% Glycerol.
- Size** ♦ 10 µg.
- Concentration** ♦ 0.1 µg/µL.
- Activity** ♦ The specific activity of Akt1 was determined to be 103 nmol/min/mg using a synthetic peptide substrate (see Activity Assay Protocol).
- Storage** ♦ This product is stable at ≤ -70 °C for up to 1 year from the date of receipt. For optimal storage, aliquot into smaller quantities after centrifugation and store at recommended temperature.
♦ **Avoid repeated freeze-thaw cycles.**

Akt1

Akt1, also known as PKB α , is a serine/threonine kinase that belongs to the Akt family. Akt1 is activated in cells in response to diverse stimuli such as hormones, growth factors and extracellular matrix components and is involved in glucose metabolism, transcription, survival, cell proliferation, angiogenesis, and cell motility (1). Akt1 is frequently over-expressed and active in many types of human cancers including cancers of the colon, breast, brain, pancreas, and prostate as well as lymphomas and leukemias (2).

References

1. Coffey, P.J. *et al.* (1998) *Biochem J.* **335**:1.
2. Anderson, K.E. *et al.* (1998) *Curr Biol.* **8**:684.

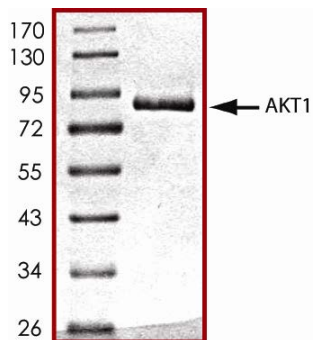


Figure 1: The approximate molecular weight is 85 kDa and the purity is > 95%.

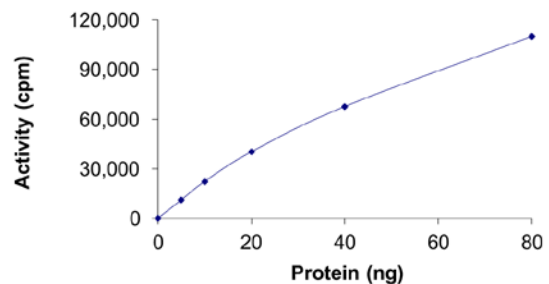


Figure 2: Enzymatic assay results. The specific activity of Akt1 was determined to be 103 nmol/min/mg as per activity assay protocol (on reverse).

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Activity Assay Protocol

Solutions Required

- **Active Kinase** - Active Akt1 (0.1 µg/µL) diluted with Kinase Dilution Buffer V and assayed as outlined in Figure 2.
Note: These are suggested working dilutions. Optimal dilutions should be determined by each laboratory for each application.
- **Kinase Assay Buffer I, pH 7.2** - 25 mM MOPS, 12.5 mM β-glycerolphosphate, 25 mM MgCl₂, 5 mM EGTA, 2 mM EDTA. Add 0.25 mM DTT to the Kinase Assay Buffer I prior to use.
- **Kinase Dilution Buffer V, pH 7.2** - Kinase Assay Buffer I diluted at a 1:4 ratio (5X dilution) with 5% Glycerol solution.
- **10 mM ATP Stock Solution** - Prepare the ATP Stock Solution by dissolving 55 mg of ATP in 10 mL of Kinase Assay Buffer I. Store in 200 µL aliquots at ≤ -20 °C.
- **[³³P]-ATP Assay Cocktail** - Prepare 250 µM [³³P]-ATP Assay Cocktail in a designated radioactive work area by combining 150 µL of 10 mM ATP Stock Solution, 100 µL of [³³P]-ATP (1 mCi/100 µL), and 5.75 mL of Kinase Assay Buffer I. Store in 1.0 mL aliquots at ≤ -20 °C.
- **Substrate** - Akt-sub synthetic peptide substrate (CKRPRAASFAE) diluted in 25 mM Tris-HCl buffer (pH 7.5) to a final concentration of 1.0 mg/mL.

Assay Procedure

1. Thaw the [³³P]-ATP Assay Cocktail in a shielded container in a designated radioactive work area.
2. Thaw the Active Akt1, Kinase Assay Buffer I, Substrate, and Kinase Dilution Buffer V on ice.
3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20 µL.

Reaction Component	Amount
Diluted Active Akt1	10 µL
Substrate (1.0 mg/mL Stock Solution)	5.0 µL
Distilled Water (4 °C)	5.0 µL

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 or deionized water.
5. Initiate the reaction by the addition of 5.0 µL [³³P]-ATP Assay Cocktail, bringing the final volume up to 25 µL. 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 (add 10 mL of phosphoric acid and make a 1-liter solution with distilled or deionized water) with constant gentle stirring. It is recommended that the strips be washed a total of three times for approximately 10 minutes each.
8. Count the radioactivity (cpm) 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.

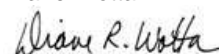
Calculation of [³³P]-ATP Specific Activity (SA) (cpm/pmol)

Specific Activity (SA) = cpm for 5.0 µL [³³P]-ATP/pmole of ATP (in 5.0 µL of a 250 µM ATP stock solution, i.e. 1250 pmoles)

Calculation of Kinase Specific Activity (SA) (pmol/minutes/µg or nmol/minutes/mg)

Corrected cpm from reaction / [(SA of ³³P-ATP in cpm/pmol) x (Reaction time in minutes) x (Enzyme amount in µg or mg)] x [(Reaction volume) / (Spot Volume)]

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Quality & Regulatory Affairs