

## Product: AKT2, Active

Catalog #: 02-2002

Amount: 5µg

### Product Description

Recombinant full-length human AKT2 was expressed by baculovirus in Sf9 insect cells using a N-terminal GST tag. The gene accession number is [NM\\_001626](#)

### Gene Aliases

PRKBB; PKBBETA; RAC-BETA

### Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.25mM DTT, 0.1mM EGTA, 0.1mM EDTA, 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.

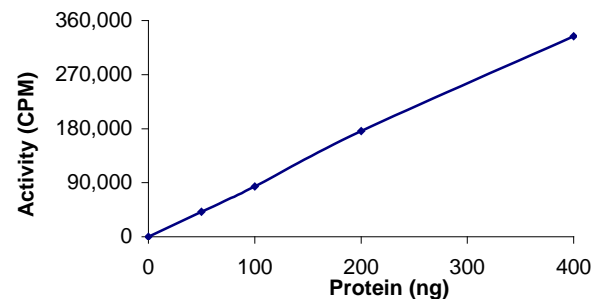
### Scientific Background

AKT2 or Protein Kinase B β (PKBβ) is a serine/threonine kinase that is a member of the AKT family. AKT2 like the other AKT members 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). The PI3K generates phosphatidylinositol-3,4,5-trisphosphate (PIP<sub>3</sub>), a lipid second messenger essential for the translocation of AKT2 to the plasma membrane where it is phosphorylated and activated by phosphoinositide-dependent kinase-1 (PDK-1) (2).

### References

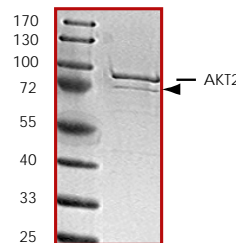
1. Coffey, P.J. et al: Protein kinase B (c-Akt): a multifunctional mediator of phosphatidylinositol 3-kinase activation. *Biochem J.* 1998 Oct 1; 335 ( Pt 1):1-13.
2. Anderson, K.E. et al: Translocation of PDK-1 to the plasma membrane is important in allowing PDK-1 to activate protein kinase B. *Curr Biol.* 1998 Jun 4;8(12): 684-91.

### Specific Activity



The specific activity of AKT2 was determined to be **46 nmol/min/mg** as per activity assay protocol.

### Purity



The purity was determined to be **>90%** by densitometry. Approx. MW **85kDa**.

## AKT2, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog Number 02-2002  
 Quantity 5µg  
 Specific Activity 46 nmol/min/mg  
 Specific Lot Number K089-3

Purity >90%  
 Format 5µg in 50µl  
 Concentration 0.1µg/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.

This product is sold for laboratory research use or further manufacturing only and should not be used for human therapeutic or diagnostic applications. The information presented is believed to be accurate; however, said information and products are offered without warranty or guarantee since the ultimate conditions of use and the variability of the materials treated are beyond our control. Nothing disclosed herein is to be construed as a recommendation to use our products in violation of any patents. Under no circumstances shall ARP American Research Products, Inc. be liable for damages, whether consequential, compensatory, incidental or special, strict liability or negligence, breach of warranty or any other theory arising out of the use of the products available from ARP American Research Products, Inc. Nothing contained herein warrants that the use of the products will not infringe on the claims of any patents covering the product itself or the use thereof in combination with other products or in the operation of any process.



## Activity Assay Protocol

### Reaction Components

#### Active Kinase (Catalog #: 02-2002)

Active AKT2 (0.1µg/µl) diluted with Kinase Dilution Buffer (Catalog #: K23-09) 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 AKT2 for optimal results).

#### Kinase Dilution Buffer, pH7.2 (Catalog #: K23-09)

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

#### Kinase Assay Buffer, pH7.2 (Catalog #: K01-09)

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

#### [<sup>32</sup>P]-ATP Assay Cocktail

Prepare 250µM [<sup>32</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>32</sup>P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer (Catalog #: K01-09). Store 1ml aliquots at -20°C.

#### 10mM ATP Stock Solution, pH7.2 (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer (Catalog #: K01-09). Store 200µl aliquots at -20°C.

#### Substrate

Synthetic peptide substrate (CGRTGRRNSI-acid) diluted in distilled water to a final concentration of 1mg/ml.

### Assay Protocol

- Step 1. Thaw [<sup>32</sup>P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2. Thaw the Active AKT2, Kinase Assay Buffer, Substrate and Enzyme 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 AKT2 (Catalog #02-2002).
  - Component 2. 5µl of 1mg/ml stock solution of substrate
  - 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>32</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>32</sup>P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5µl [<sup>32</sup>P]-ATP / pmoles of ATP (in 5µl of a 250µM ATP stock solution, i.e., 1250 pmoles)

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

Corrected cpm from reaction / [(SA of <sup>32</sup>P-ATP in cpm/pmol)\*(Reaction time in min)\*(Enzyme amount in µg or mg)]\*[(Reaction Volume) / (Spot Volume)]

This product is sold for laboratory research use or further manufacturing only and should not be used for human therapeutic or diagnostic applications. The information presented is believed to be accurate; however, said information and products are offered without warranty or guarantee since the ultimate conditions of use and the variability of the materials treated are beyond our control. Nothing disclosed herein is to be construed as a recommendation to use our products in violation of any patents. Under no circumstances shall ARP American Research Products, Inc. be liable for damages, whether consequential, compensatory, incidental or special, strict liability or negligence, breach of warranty or any other theory arising out of the use of the products available from ARP American Research Products, Inc. Nothing contained herein warrants that the use of the products will not infringe on the claims of any patents covering the product itself or the use thereof in combination with other products or in the operation of any process.