Affinity-Purified Sheep Anti-human/mouse/rat Akt1 Antibody

ORDERING INFORMATION

Catalog Number: AF17751

Lot Number: ZIF01

Size: 100 μg (sufficient for 100 mL of blotting solution)

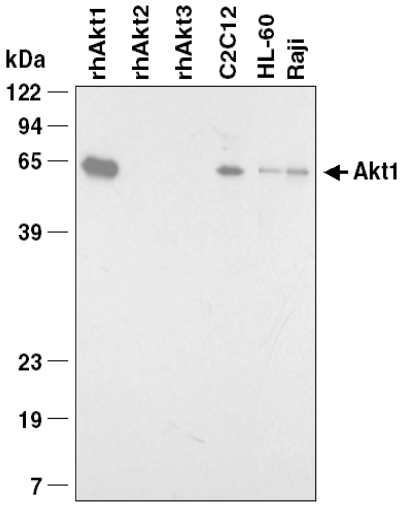
Storage: -20° C

Specificity: human, mouse, and rat Akt1

Immunogen: *E. coli*-derived rhAkt1 (aa 1 - 149)

Ig Type: sheep IgG

Application: Western blot



Detection of Akt1 with AF17751. Recombinant human Akt1, Akt2, and Akt3 (rhAkt1-3; 2 ng each), and lysates of mouse C2C12 and human HL-60 and Raji cells were resolved by SDS-PAGE. Following electrophoresis, lysates and recombinant proteins were transferred to an Immobilon-P membrane and immunoblotted with 1.0 μg/mL anti-Akt1, as described in *Protocols for Immunoblotting*.

Background

The serine/threonine kinase Akt, also known as protein kinase B (PKB), is a central regulator of such diverse cellular processes as glucose uptake, cell cycle progression, and apoptosis. In mammals, three highly homologous members define the Akt family: Akt1 (PKBα), Akt2 (PKBβ), and Akt3 (PKBγ). Akt1 is the most ubiquitously expressed family member. All three Akts contain an amino-terminal pleckstrin homology domain, a central kinase domain, and a carboxyl-terminal regulatory domain.

Preparation

Sheep antibodies were raised against purified, *E. coli*-derived recombinant human v-akt murine thymoma viral oncogene homolog 1 (rhAkt1; aa 1 - 149; Accession # P31749). Polyclonal antibody was affinity-purified on a column derivatized with the recombinant protein and further purified by isolating the IgG fraction.

Formulation

Lyophilized from a 0.2 μm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Reconstitution

Reconstitute in PBS containing 0.02% NaN_3.

Storage

Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C **in a manual defrost freezer** for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

Specificity

This antibody detects endogenous human, mouse, and rat Akt1 at 60 kDa using Western blot. The antibody is specific for Akt1 and does not detect recombinant Akt2 or Akt3.

Application

Western blot - An antibody concentration of 1.0 μg/mL is recommended.

Protocols for Immunoblotting

Blotting Buffer	Blocking Solution	Antibody Solution
25 mM Tris, pH 7.4	5% nonfat dry milk	5% nonfat dry milk
0.15 M NaCl	in Blotting Buffer	in Blotting Buffer
0.1% Tween® 20	Adjust pH to 7.4	Adjust pH to 7.4

1. Transfer the electrophoresed proteins to Immobilon-P membrane (Millipore) and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane overnight at 4° C in Antibody Solution containing 1.0 μg/mL anti-human/mouse/rat Akt1.
3. Wash the membrane at room temperature for 1 hour with 5 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane at room temperature for 1 hour in Antibody Solution containing a 1:1,000 dilution of HRP-conjugated donkey anti-sheep IgG (R&D Systems, Catalog # HAF016).
5. Wash the membrane for 1 hour with 5 or more changes of Blotting Buffer.
6. Detect with WesternGlo™ Chemiluminescent Detection Reagent (R&D Systems, Catalog # AR004) or equivalent.

Cell lysates for Western blottings - To prepare total cell lysates, cells are solubilized in hot 2x SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, 10 mM NaF, and bromophenyl blue) at 2 x 10^6 - 1 x 10^7 cells per mL. The extracts are heated in a boiling water bath for 5 minutes and then sonicated with a probe sonicator with 3 - 4 bursts of 5 - 10 seconds each.

Optimal dilutions should be determined by each laboratory for each application.

R&D Systems, Inc.
1-800-343-7475

6/27/07