Akt Isoform Antibody Sampler Kit

1 Kit
(4 x 20 µl)

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Description: Akt Isoform Specific Antibody Sampler Kit provides an economical means of distinguishing between the three Akt isoforms, Akt1, Akt2 and Akt3, as well as a pan-Akt antibody. The kit provides enough primary and secondary antibodies to perform two Western blot experiments.

Background: Akt, also referred to as PKB or Rac, plays a critical role in controlling survival and apoptosis (1-3). This protein kinase is activated by insulin and various growth and survival factors to function in a wortmannin-sensitive pathway involving PI3 kinase (2,3). Akt is activated by phospholipid binding and activation loop phosphorylation at Thr308 by PDK1 (4) and by phosphorylation within the carboxy terminus at Ser473. The previously elusive PDK2 responsible for phosphorylation of Akt at Ser473 has been identified as mammalian target of rapamycin (mTor) in a rapamycin-insensitive complex with rictor and Sin1 (5,6). Akt promotes cell survival by inhibiting apoptosis by phosphorylating and inactivating several targets, including Bad (7), forkhead transcription factors (8), c-Raf (9) and phospholipid binding and activation loop phosphorylation of the PI3 kinase/Akt signaling pathway (10). LY294002 is a specific PI3 kinase inhibitor (11).

Another essential Akt function is the regulation of glycogen synthesis machinery due to inactivation of its effector, p70 S6 kinase and activation of the eukaryotic initiation factor 4E binding protein 1 (4E-BP1), an inhibitor of translation (18,19).

Specificity/Sensitivity: Each isoform-specific antibody recognizes endogenous levels of the specified Akt isoform independent of its phosphorylation state. Neither antibody cross-reacts with the two other isoforms. Akt Antibody recognizes endogenous levels of all three Akt isoforms.

Source/Purification: Each isoform-specific antibody is produced by immunizing mice or rabbits with a synthetic peptide that is specific for the Act isoform. Akt1 monoclonal antibody is produced by immunizing animals with a synthetic peptide surrounding Leu110 of human Akt1. Akt2 monoclonal antibody #3063 is produced by immunizing rabbits with a synthetic peptide corresponding to residues of human Akt2. Akt3 monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Arg116 of human Akt3 protein. Akt(pan) monoclonal antibody is produced by immunizing animals with a synthetic peptide at the carboxy-terminal sequence of mouse Akt.

Background References:

For Research Use Only. Not For Use In Diagnostic Procedures.
Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. 20X Phosphate Buffered Saline (PBS): (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH2O, mix.
2. 10X Tris Buffered Saline (TBS): (#12496) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH2O, mix.
3. 1X SDS Sample Buffer: Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH2O.
4. 10X Tris-Glycine SDS Running Buffer: (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH2O, mix.
5. 10X Tris-Glycine Transfer Buffer: (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH2O, mix.
6. 10X Tris Buffered Saline with Tween® 20 (TBST): (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH2O, mix.
7. Nonfat Dry Milk: (#9999)
8. Blocking Buffer: 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. Wash Buffer: (#9997) 1X TBST
10. Bovine Serum Albumin (BSA): (#9998)
11. Primary Antibody Dilution Buffer: 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
12. Biotinylated Protein Ladder: (#7727)
13. Prestained Protein Marker, Broad Range (Premixed Format): (#7720)
14. Blotting Membrane and Paper: (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. Secondary Antibody Conjugated to HRP: anti-rabbit (#7074); anti-mouse (#7076)
16. Detection Reagent: Lumiglo® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl for a 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). NOTE: Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrophoresis and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electotransfer to nitrocellulose membrane (#12369).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking

1. Optional) After transfer, wash nitrocellulose membrane with 25 ml TBST for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. Incubate membrane with 10 ml Lumiglo® (0.5 ml 20X Lumiglo® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.