Revision 3

Store at -20C

#9273

Akt Control Cell Extracts

Controls for 10 western blots

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Product Includes	Product #	Quantity
Akt Control Cell Extracts (Jurkat +Calyculin A)	48217	200 µl
Akt Control Cell Extracts (Jurkat + LY294002)	66376	200 µl

Description

Phosphorylated Akt Cell Extracts: Total cell extracts from Jurkat cells, serum starved overnight and then treated with Calyculin A (CST #9902) to preserve their activated Akt state, serve as a positive control. Supplied in SDS Sample Buffer.

Nonphosphorylated Akt Cell Extracts: Total cell extracts from Jurkat cells, serum starved overnight and then treated with 50 µM LY294002 (CST #9901) for one hour, serve as a negative control. Supplied in SDS Sample Buffer.

Storage

Supplied in SDS Sample Buffer: 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red.
Store at –20°C, or at –80°C for long-term storage.

Background

Akt, also referred to as PKB or Rac, plays a critical role in controlling cell 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 through phosphorylation and inactivation of several targets, including Bad (7), forkhead transcription factors (8), c-Raf (9), and caspase-9. PTEN phosphatase is a major negative regulator of the PI3K/Akt signaling pathway (10). LY294002 is a specific PI3 kinase inhibitor (11). Another essential Akt function is the regulation of glycogen synthesis through phosphorylation and inactivation of GSK-3α and β (12,13). Akt may also play a role in insulin stimulation of glucose transport (12). In addition to its role in survival and glycogen synthesis, Akt is involved in cell cycle regulation by preventing GSK-3β-mediated phosphorylation and degradation of cyclin D1 (14) and by negatively regulating the cyclin-dependent kinase inhibitors p27 Kip1 (15) and p21 Waf1/Cip1 (16). Akt also plays a critical role in cell growth by directly phosphorylating mTOR in a rapamycin-sensitive complex containing raptor (17). More importantly, Akt phosphorylates and inactivates tuberin (TSC2), an inhibitor of mTOR within the mTOR-raptor complex (18,19).

Directions for Use

Boil for 3 minutes prior to use. Load 20 µl of phosphorylated and nonphosphorylated Akt extracts per lane.

Background References

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