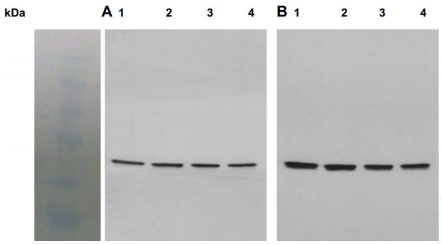
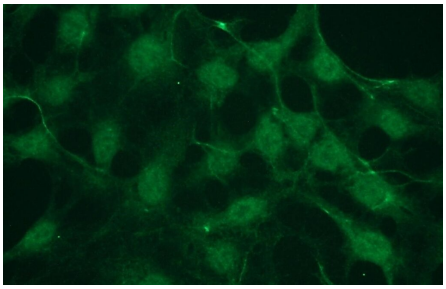
Product Data Sheet: Phospho-Akt1 (Ser473)

Cat. No:	ABP-0637
Conjugate:	Unconjugated
Size:	100 ug
Clone:	Poly
Concentration:	1mg/ml
Host:	Rb
Isotype:	IgG
Reactivity:	Hu, Ms, Rt
Applications:	Western Blot: 1:1000 Immunohistochemistry: 1:100 Immunoprecipitation: 1:50 Immunocytochemistry: 1:50
Synonyms:	AKT;CWS6;PKB;PKB-ALPHA;PRKBA;RAC;RAC-ALPHA;AKT1
Background:	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 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 PI3 kinase/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-3 and (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-3 -mediated phosphorylation and degradation of cyclin D1 (14) and by negatively regulating the cyclin dependent kinase inhibitors p27 Kip (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). Specificity/Sensitivity: Phospho-Akt1 (Ser473) (D7F10) Rabbit mAb (Akt1 Specific) recognizes endogenous levels of Akt1 protein only when phosphorylated at Ser473. It does not detect Akt2 protein when phosphorylated at Ser474.
Form:	liquid
Buffer:	20 mM Tris-HCl, pH 8.0, 10 mg/ml BSA, 0.05% Sodium Azide
Storage:	Short term at 2-4°C, longer term-20°C. Avoid repeated freezing and thawing



A) Western blot analysis of Akt activation in striatal neurons stimulated with DHPG (mGluR5 agonist) for 0 (lane 1), 2 (lane 2), 5 (lane 3) or 10 (lane 4) min. (B) Western blot analysis of total Akt (detected with anti-Akt1 antibody, in striatal neurons stimulated with DHPG (a mGluR5 agonist) for 0 (lane 1), 2 (lane 2), 5 (lane 3) or 10 (lane 4) min. Wells were equally loaded with 100 µg of whole cell lysate proteins.



Representative picture of Akt1 (only when phosphorylated at the Ser-473 residue) in HEK293 cells, visualized with clonal rabbit anti-Akt1, pSer-473 monospecific antibody. Primary antibody dilution – 1:50.

WESTERN BLOT (WB) PROTOCOL - INSTRUCTION MANUAL Western immunoblotting solutions:

Wash buffer: 1x Tris Buffered Saline (TBS); 0.1% Triton X-100 –
Blocking buffer: 1xTBS; 0.1% Triton X-100; 2% BSA
For western blots, incubate the membrane with antibody diluted in blocking buffer for 2 hours at room temperature.

IMMUNOCYTOCHEMISTRY (ICC) PROTOCOL - INSTRUCTION MANUAL

1. Coat coverslips with 1% gelatin-coating solution for 2 hours at room temperature (RT); rinse with distilled water, and let to dry overnight. Before plating the cells, wash the coated coverslips briefly with PBS. 2. Fix the cells with 4% paraformaldehyde solution (in PBS, pH 7.2), for 15 min at RT. 3. Wash 2 x 3 min with PBS. 4. Permeabilize the cells with 0.1% Triton X-100 solution (in PBS, pH 7.2) for 5 min on ice. 5. Wash 2 x 3 min with PBS. 6. Incubate the cells in blocking buffer (0.3M glycine in PBS, 2% BSA) for 30 min at RT. 7. Incubate the cells with primary antibody: anti-Akt1, pSer-473 clonal antibody at the dilution of 1:50 – 1:100 in antibody dilution buffer (PBS, 1% BSA) for 1 hour at RT in humid chamber. 8. Wash 2 x 3 min with PBS. 9. Apply the secondary antibody (in this case, the HRP goat anti-rabbit IgG- Cat.n. IS20402 from was used at 1:300 in antibody dilution buffer, and cells were incubated for 1 hour at RT in dark). 10. Wash 3 x 3 min with PBS. 11. Rinse once with distilled water. 12. Mount the slide for observation, with a drop of anti-fade mounting medium.