IMMUNOLOGICAL SCIENCES Product Data Sheet

AB- 10521 Anti - Akt (pSer-473) – *Rabbit Polyclonal Antibody*

Size: **100 ul**
Conc: 1mg/ml

Product description: Rabbit anti-Akt, pSer-473 (recognizing the phosphorylated form of Akt1 at the pSer-473 residue, Akt2 at the pSer-474 residue, and Akt3 at the pSer-472 residue)

Immunogen: Peptide surrounding pSer-473 at the C-terminal sequence of human AKT protein

Species Reactivity: Human, mouse, rat - tested

Buffer: **Liquid in** PBS containing 50% glycerol,0.5% BSA, and 0,02% Sodium Azide

Storage: 10 µl aliquots at -20 ˚C.

Handling: Avoid repeated freezing and thawing

Applications: Western blot, Immunoprecipitation, ELISA, Immunocytochemistry (ICC)

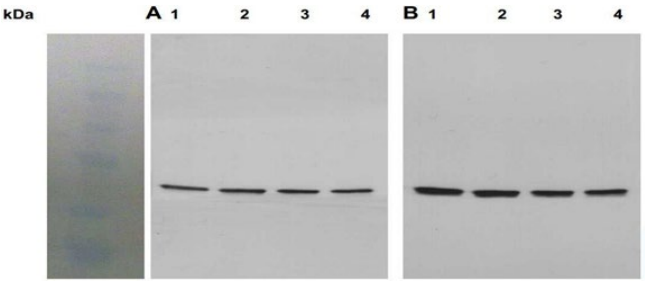
Dilution range: **Western blotting : 1:1,000 – 1: 1,500**
Immunocytochemistry: 1:500 – 1:1000

Western immunoblotting solutions:

For a dilution of 1:1,000: Take 1 ul of Abs and add 999 ul of dilution buffer

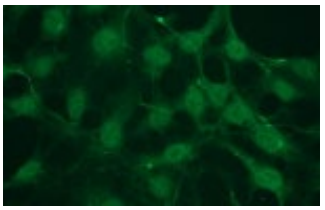
- Wash buffer: 1x Tris Buffered Saline (TBS); 0.1% Triton X-100
- Blocking buffer: 1xTBS; 0.1% Triton X-100; 2% BSA

For western blots, incubate the membrane with antibody diluted in blocking buffer 2 hours at room temperature.



A) Western blot analysis of Akt activation using AB-10521 Phospho Ser 473 AKT 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, AB-82322 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 - 1:100



AB-10521 – AKT Phosho-Ser473

Immunocytochemistry – on cells

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: phospho- AKT Ser-473 antibody at the dilution of 1:100 - 1:200 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 goat anti-rabbit IgG-FITC 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.