Anti - Akt1 (pSer-473)*

Rabbit clonal antibody

CAT#

DB 127-0.05 (50 µl)
DB 127-0.1 (100 µl)

PRODUCT INFORMATION

Clone number: X20-A
Uniprot: Human: P31749; Mouse: P31750; Rat: P47196
Product description: Rabbit anti-Akt1, pSer-473 specific clonal IgGs (recognizing the phosphorylated form of Akt1 at the pSer-473 residue, Akt2 at the pSer-474 residue, and Akt3 at the pSer-472 residue)
Basic information: Major clone of IgG obtained from the crude rabbit antiserum by in vitro cloning technology, detecting the Akt1 protein
Immunogen: Peptide surrounding pSer-473 at the C-terminal sequence of human AKT1 protein
Species Reactivity: Human, mouse, rat - tested

Buffer: 20 mM Tris-HCl, pH 8.0
Stabilizer: 10 mg/ml BSA
Preservative: 0.05% Sodium Azide

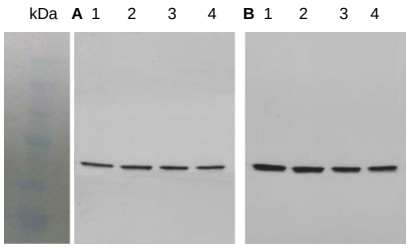
Storage: 10 µl aliquots at -20°C
Handling: Avoid repeated freezing and thawing
Expiration: 24 months from the shipping date

Applications: Western blot, Immunoprecipitation, ELISA, Immunocytochemistry (ICC)
Dilution range: Western blotting – 1:5,000
ELISA – 1:100,000 – 1:200,000

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.



Anti - Akt1, pSer-473 (DB 127)

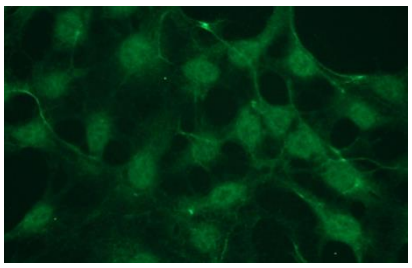
(A) Western blot analysis of Akt activation in striatal neurons stimulated with DHPG (mGluR5 agonist) for 0 (lane 1), 2 (lane 2), 5 (lane 3) or 10 (lane 4) min.

(B) Western blot analysis
of total Akt (detected with anti-Akt1 antibody, DB 126) 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.

Western blot was performed by Dr. Fabiola M. Ribeiro, Departamento de Bioquimica e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Brasil.

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 **1:200 - 1:500** 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 from Jackson Immunoresearch, cat. # 111-095-003, 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.



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 - 1:300.

PRECAUTIONS

1. Intended for professional In Vitro Diagnostic use in laboratories.
2. Do not use after expiration date stamped on vial label.
3. Avoid contamination of the reagent.
4. Any discrepancies in the recommended procedures stated in the working protocol may affect the final results.
5. The reagent contains sodium azide (NaN_3) which is highly toxic in higher concentrations. The concentration in the reagent (0.05%) is not considered as hazardous.
6. Disposal of waste material must be conducted in accordance with local regulations.
7. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.

DB Biotech, Inc., Popradska 80, 040 11 Kosice, Slovak Republic
Tel.: +421 55 789 0010, Fax: +421 55 789 0024, E-mail: info@dbbiotech.com, www.dbbiotech.com
Revision date: 17.01.2017