

Anti - AKT3

Rabbit clonal antibody

CAT#

DB 184-0.05 (50 µl)
DB 184-0.1 (100 µl)

PRODUCT INFORMATION

Clone number: E18-H
Uniprot: Human: Q9Y243; Mouse: Q9WUA6; Rat: Q63484
Product description: Rabbit anti- AKT3 clonal IgGs

Basic information: Major clone of IgG obtained from the crude rabbit antiserum by in vitro cloning technology, detecting specifically the Akt3 protein

Immunogen: Peptide derived from the C-terminal sequence of human AKT3 protein. Antibody recognizes the epitope located between Tyr452 - Pro466.

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 (IP), ELISA, Immunocytochemistry (ICC)

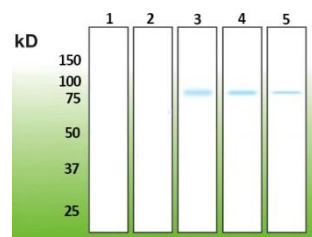
Dilution range: Western blotting – 1:500 to 1:1,000
ELISA – 1:100,000 – 1:200,000

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; 8% skim milk

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

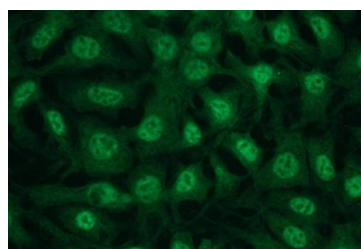


Anti - AKT3 (DB 184)

Western blot analysis of DB 184 specificity using recombinant proteins AKT1, AKT2 and AKT3: lane 1 – 0.2 µg of human His-AKT1; lane 2 -0.2 µg of human GST-AKT2; lane 3 – 1 µg of human GST-AKT3, lane 4 – 0.5 µg of human GST-AKT3, lane 5 – 0.2 µg of human GST-AKT3 loaded.

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-Akt3 clonal antibody at the dilution of 1:300 - 1:1000 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 Akt3 expression in HEK293 cells, visualized with clonal rabbit anti-Akt3 monospecific antibody. Primary antibody dilution - 1:500.

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 (NaN₃) 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.