

## Anti - PKC- $\zeta$

### Rabbit clonal antibody

#### CAT#

DB 011-0.05 (50  $\mu$ l)  
DB 011-0.1 (100  $\mu$ l)

#### PRODUCT INFORMATION

**Clone number:** R14-K  
**Uniprot:** Human: Q05513; Mouse: Q02956; Rat: P09217  
**Product description:** Rabbit anti-PKC- $\zeta$  clonal IgGs  
**Basic information:** Major clone of rabbit immunoglobulin corresponding to immunogenic peptide  
**Immunogen:** Peptide derived from internal sequence on human PKC- $\zeta$ . Antibody recognizes the epitope located between Lys220 - Leu231.  
**Species reactivity:** Human, mouse, rat - tested

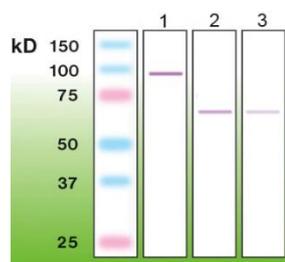
**Buffer:** 20 mM Tris-HCl, pH 8.0  
**Stabilizer:** 10 mg/ml BSA  
**Preservative:** 0.05% Sodium Azide  
**Storage:** 10  $\mu$ l aliquots at -20°C  
**Handling:** Avoid repeated freezing and thawing  
**Expiration:** 24 months from the shipping date  
**Applications:** Western blot, Immunoprecipitation, ELISA, Immunocytochemistry (ICC)  
**Dilution range:** Western blotting – 1:5,000  
ELISA – 1:20,000-1:100,000  
Immunoprecipitation – to be tested by user

#### WESTERN BLOT (WB) PROTOCOL - INSTRUCTION MANUAL

##### Western immunoblotting solutions:

- Wash buffer: 1x Tris Buffered Saline (TBS); 0.2% Triton X-100
- Blocking buffer: 1xTBS; 0.2% Triton X-100; 5% nonfat dry milk

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

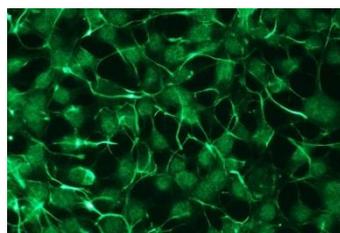


#### Anti - PKC- $\zeta$ (DB 011)

Western blot of PKC- $\zeta$  lane 1 – 50ng of recombinant human PKC- $\zeta$  (N-term GST PKC- $\zeta$ ; BML-SE443-0005); lane 2 – mouse brain crude protein lysate (100  $\mu$ g); lane 3 – rat brain crude protein lysate (100  $\mu$ g)

#### 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-PKC- $\zeta$  clonal antibody at the dilution of **1:100 - 1:400** 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 PKC- $\zeta$  expression in HEK293 cells, visualized with clonal rabbit anti-PKC- $\zeta$  monospecific antibody. Primary antibody dilution - 1:200.

#### 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<sub>3</sub>) 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.