Anti - PHOSPHO-Erk 1,2

Rabbit clonal antibody

CAT#

DB 013-0.05 (50 µl)
DB 013-0.1 (100 µl)

PRODUCT INFORMATION

Clone number: G15-B
Uniprot: Human: P27361/P28482; Mouse: Q63844/P63085; Rat: P21708/P63086
Product description: Rabbit anti - phospho - Erk 1,2 clonal IgGs
Basic information: Major clone of IgGs representing both, Erk1 and Erk2, obtained from immunoglobulins corresponding to pT-E-pY dual phosphorylation sequence
Immunogen: Peptide derived from the protein area including conserved pT-E-pY motif of activated Erk 1,2
Species reactivity: Human, mouse, rat - tested

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

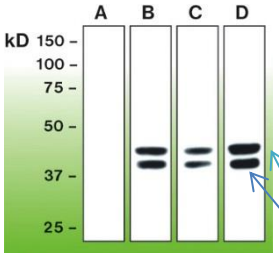
Storage: 10 µl aliquots at -20°C
Handling: Avoid repeated freezing and thawing
Expiration: 24 months from the shipping date

Applications: Western blot, Immunoprecipitation, ELISA, Immunocytochemistry (ICC)
Dilution range: Western blotting – 1:5,000
ELISA – 1:20,000-1:100,000
Immunoprecipitation – to be tested by user

WESTERN BLOT (WB) PROTOCOL - INSTRUCTION MANUAL

Western immunoblotting solutions:
- Wash buffer: 1x Tris Buffered Saline (TBS); 0.1% Triton X-100
- Blocking buffer: 1xTBS; 0.1% Triton X-100; 5% BSA (used with the primary antibody)

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

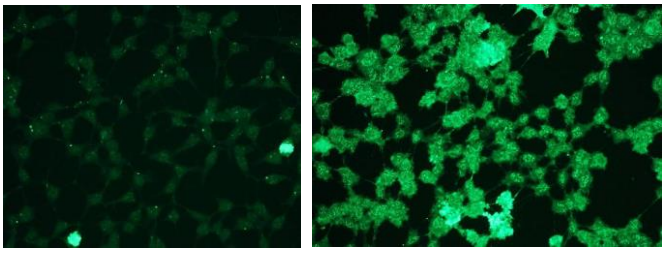


Anti - phospho-Erk 1,2 (DB 013)

Western blot analysis of Erk 1,2 activation in untreated PC12 cells (A), cells treated with EGF-100 ng/ml, 5min (B), PMA-100nM, 30min (C) in serum free DMEM, and EGF-100 ng/ml, 5min with 10% FBS in DMEM (D). Wells were equally loaded with 50 µg of whole cell lysate proteins/well

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-phospho Erk 1,2 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 pictures of phospho-Erk 1,2 expression in HEK293 cells, (**picture A** – untreated cells, **picture B** - hydrogen peroxide treated cells) visualized with clonal rabbit anti-phospho-Erk 1,2 monospecific antibody. Primary antibody dilution - 1:100.

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.

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