Anti - Erk 1,2

Rabbit clonal antibody

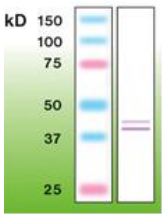
CAT#

DB 012-0.05 (50 µl)
DB 012-0.1 (100 µl)

WESTERN BLOT (WB) PROTOCOL

Western immunoblotting solutions:
- Wash buffer: 1x Tris Buffered Saline (TBS); 0.2% Triton X-100
- Blocking buffer: 1xTBS; 0.2% Triton X-100; 8% nonfat dry milk

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



Anti - Erk 1,2 (DB 012)

Western blot of Erk 1,2 in rat brain crude lysate (100 µg of protein loaded)

ERK 1,2

PRODUCT INFORMATION

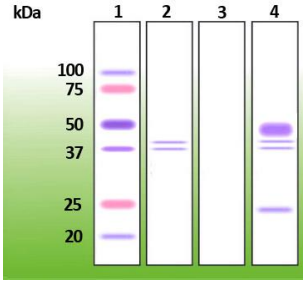
Clone number:	B20-U
Uniprot:	Human: P27361/P28482; Mouse: Q63844/P63085; Rat: P21708/P63086
Product description:	Rabbit anti - Erk 1,2 clonal IgGs
Basic information:	Major clone of IgGs representing both, Erk1 and Erk2 obtained from immunoglobulins corresponding to immunogenic peptide
Immunogen:	Peptide derived from C-terminal sequence of human Erk2. Antibody recognizes the epitope located between Ile319 - Tyr333.
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 – 1:5,000 ELISA – 1:20,000-1:100,000 Immunoprecipitation – to be tested by user

IMMUNOPRECIPITATION (IP) PROTOCOL - INSTRUCTION MANUAL

1. Dilute sample (200 - 500 µg of total protein) with Correction Buffer (2.5% v/v Nonidet P-40, 5% w/v sodium deoxycholate, 0.5% w/v SDS in ddH2O) in ratio 4:1 (v/v).
2. Add 5 µl of DB012 (anti-Erk 1,2, clone B20-U) rabbit clonal monospecific antibody, mix gently and incubate for 1 hour on ice.
3. Mix with 50 µl of ProteinG-Sepharose (washed with 10mM Tris-HCl, pH7.5*) and incubate for 30 minutes at 4°C with gentle shaking.

   *NOTE: Washing of ProteinG-Sepharose with 10mM Tris-HCl, pH7.5: Resuspend 50 µl of ProteinG-Sepharose in 1 ml of 10mM Tris-HCl, pH7.5 by precise inverting of the tube several times. Centrifuge for 1 min, 900xg at 4°C and discard supernatant. Avoid of wasting ProteinG-Sepharose agarose gel beads during discarding. Repeat this procedure for 3 times.
4. Centrifuge ProteinG-Sepharose immunocomplex for 2 min, 900xg at 4°C and discard supernatant. Wash the pellet 3 times with 1 ml of RIPA Buffer (10mM TRIS-HCl, pH7.5, 140mM NaCl, 1% v/v Nonidet P-40, 1% w/v sodium deoxycholate, 0.5% w/v SDS v ddH_2O).

   IMPORTANT: Avoid of wasting/discarding ProteinG-Sepharose immunocomplex.
5. Wash sediment with 1 ml of 10mM TRIS-HCl, pH7.5, centrifuge sample (agarose gel beads) for 1 min, 900xg at 4°C and discard the supernatant.
6. Dissociate immunocomplex from ProteinG-Sepharose with the help of Reduction Buffer (125mM TRIS-HCl, pH6.8, 3.3% SDS, 5% β-mercaptoethanol). Mix the sample with 30 µl of Reduction Buffer, shake gently and incubate for 5 minutes at 65°C.
7. Centrifuge at 3000xg for 5 minutes and transfer the supernatant (immunoprecipitated proteins) to new tube.
8. Separate the immunoprecipitated protein by 1D SDS-PAGE.

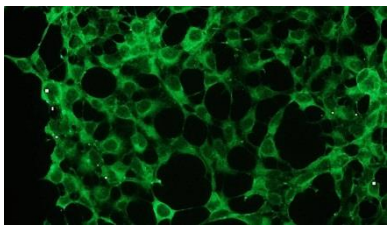


Representative picture of Erk 1,2 immunoprecipitated from HEK 293 cells, visualized with clonal rabbit anti- Erk 1,2 monospecific antibody (DB012) in Western blot analysis. Primary antibody dilution - 1:5,000.

lane 1 – molecular weight marker; lane 2 – positive control, Western blot of HEK 293 (100 µg) with anti–Erk 1, 2 rabbit clonal primary antibody; lane 3 – negative control, immunoprecipitation without primary antibody; lane 4 – immunoprecipitated Erk 1,2 (~40 and ~45 kDa proteins) from HEK 293 cells (500 µg of crude protein extract) using DB012 antibody

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-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 picture of Erk 1,2 expression in HEK293 cells, visualized with clonal rabbit anti-Erk 1,2 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_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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