Anti-Phospho-Erk1 (T202 + Y204) + Erk2 (T185 + Y187) Antibody [SC58-01]

ET1610-13

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Cell, IF-Tissue, FC, IP
Molecular Wt:	Predicted band size: 41/43 kDa
Clone number:	SC58-01

Description: The activation of signal transduction pathways by growth factors, hormones and neurotransmitters is mediated through two closely related MAP kinases, p44 and p42, designated extracellular-signal related kinase 1 (ERK 1) and ERK 2, respectively. ERK proteins are regulated by dual phosphorylation at Tyrosine 204 and 187 and Threonine 177 and 160 residues mapping within a characteristic Thr-Glu-Tyr motif. Phosphorylation at both the Threonine 202 and Tyrosine 204 residues of ERK1 and Threonine 185 and Tyrosine 187 residues of ERK2 is required for full enzymatic activation. The structural consequences of dual-phosphorylation in the ERK2 include active site closure, alignment of key catalytic residues that interact with ATP, and remodeling of the activation loop. In response to activation, MAP kinases phosphorylate downstream components on serine and threonine. Upstream MAP kinase regulators include MAP kinase kinase (MEK), MEK kinase and Raf-1. The ERK family has three additional members: ERK 3, ERK 5 and ERK 6.

Immunogen: Synthetic peptide within Human aa 166-215 / 379.

Positive control: SH-SY5Y cell lysate, SH-SY5Y treated with 100ng/mL hβ-NGF for 10 minutes cell lysate, PC-12 treated with 100ng/mL hβ-NGF for 10 minutes cell lysate, SK-Br-3 whole cell lysate, NIH/3T3 cell lysate, NIH/3T3 treated with 200nM PMA for 30 minutes cell lysate, HeLa treated with 200nM PMA for 30 minutes cell lysate, human thyroid carcinoma tissue.

Subcellular location: Cytoplasm, Nucleus.

Database links: SwissProt: P27361 Human | P28482 Human | P63085 Mouse | Q63844 Mouse | P21708 Rat | P63086 Rat

Recommended Dilutions:

WB	1:5,000-1:10,000
IF-Cell	1:100
IHC-P	1:1,000
IF-Tissue	1:200
FC	1:1,000
IP	Use at an assay dependent concentration.

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

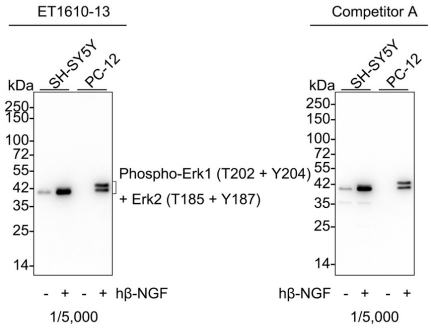

Fig1: Western blot analysis of Phospho-Erk1 (T202 + Y204) + Erk2 (T185 + Y187) on different lysates with Rabbit anti-Phospho-Erk1 (T202 + Y204) + Erk2 (T185 + Y187) antibody (ET1610-13) at 1/5,000 dilution and competitor's antibody at 1/5,000 dilution.

Lane 1: SH-SY5Y cell lysate
Lane 2: SH-SY5Y treated with 100ng/mL hβ-NGF for 10 minutes cell lysate
Lane 3: PC-12 cell lysate
Lane 4: PC-12 treated with 100ng/mL hβ-NGF for 10 minutes cell lysate

Lysates/proteins at 15 µg/Lane.

Predicted band size: 41/43 kDa
Observed band size: 41/43 kDa

Exposure time: 1 minute 50 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1610-13) at 1/5,000 dilution and competitor's antibody at 1/5,000 dilution were used in 5% BSA at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

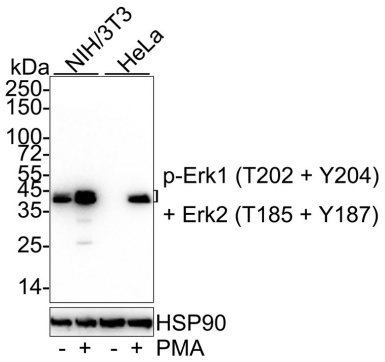

Fig2: Western blot analysis of Phospho-Erk1 (T202 + Y204) + Erk2 (T185 + Y187) on different lysates with Rabbit anti-Phospho-Erk1 (T202 + Y204) + Erk2 (T185 + Y187) antibody (ET1610-13) at 1/5,000 dilution.

Lane 1: NIH/3T3 cell lysate
Lane 2: NIH/3T3 treated with 200nM PMA for 30 minutes cell lysate
Lane 3: HeLa cell lysate
Lane 4: HeLa treated with 200nM PMA for 30 minutes cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 41/43 kDa
Observed band size: 41/43 kDa

Exposure time: 13 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

hour at room temperature.

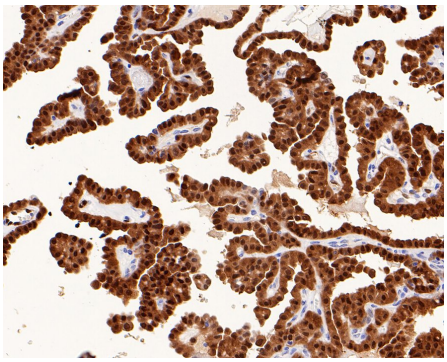

Fig3: Immunohistochemical analysis of paraffin-embedded human thyroid carcinoma tissue with Rabbit anti-Phospho-Erk1 (T202 + Y204) + Erk2 (T185 + Y187) antibody (ET1610-13) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1610-13) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

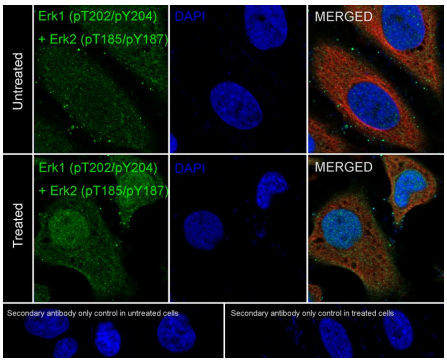

Fig4: Immunocytochemistry analysis of HeLa cells treated with 200nM PMA for 30 minutes labeling Phospho-Erk1 (T202 + Y204) + Erk2 (T185 + Y187) with Rabbit anti-Phospho-Erk1 (T202 + Y204) + Erk2 (T185 + Y187) antibody (ET1610-13) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Phospho-Erk1 (T202 + Y204) + Erk2 (T185 + Y187) antibody (ET1610-13) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

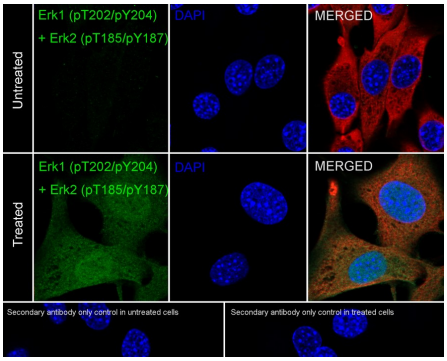

Fig5: Immunocytochemistry analysis of NIH/3T3 cells treated with 200nM PMA for 30 minutes labeling Phospho-Erk1 (T202 + Y204) + Erk2 (T185 + Y187) with Rabbit anti-Phospho-Erk1 (T202 + Y204) + Erk2 (T185 + Y187) antibody (ET1610-13) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Phospho-Erk1 (T202 + Y204) + Erk2 (T185 + Y187) antibody (ET1610-13) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

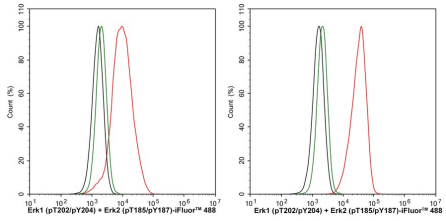

Fig6: Flow cytometric analysis of HeLa cells untreated (left) or treated (right) with PMA for 30 minutes labeling Phospho-Erk1 (T202 + Y204) + Erk2 (T185 + Y187).

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1610-13, 1μg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Wang ZH et al. Sunitinib mesylate inhibits proliferation of human colonic stromal fibroblasts in vitro and in vivo. J Zhejiang Univ Sci B 15:701-12 (2014).
2. Assi J et al. Transglutaminase 2 overexpression in tumor stroma identifies invasive ductal carcinomas of breast at high risk of recurrence. PLoS One 8:e74437 (2013).