Anti-ERK1/2 Antibody [SA43-03]

ET1601-29

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Zebrafish
Applications:	WB, IF-Cell, IF-Tissue, IP, FC, IHC-P, IHC-Fr
Molecular Wt:	Predicted band size: 43/41 kDa
Clone number:	SA43-03

Description: The first mitogen-activated protein kinase to be discovered was ERK1 (MAPK3) in mammals. Since ERK1 and its close relative ERK2 (MAPK1) are both involved in growth factor signaling, the family was termed "mitogen-activated". With the discovery of other members, even from distant organisms (e.g. plants), it has become increasingly clear that the name is a misnomer, since most MAPKs are actually involved in the response to potentially harmful, abiotic stress stimuli (hyperosmosis, oxidative stress, DNA damage, low osmolarity, infection, etc.). Because plants cannot "flee" from stress, terrestrial plants have the highest number of MAPK genes per organism ever found[citation needed]. Thus the role of mammalian ERK1/2 kinases as regulators of cell proliferation is not a generic, but a highly specialized function.

Immunogen: Recombinant protein within human ERK2 aa 180-379.

Positive control: HeLa cell lysate, Jurkat cell lysate, HepG2 cell lysate, NIH/3T3 cell lysate, PC-12 cell lysate, C6 cell lysate, Jurkat, human breast carcinoma tissue, mouse esophagus tissue, mouse stomach tissue, Hela, MCF-7, NIH/3T3, A549.

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
IF-Cell	1:2,000
IF-Tissue	1:50
FC	1:1,000
IP	Use at an assay dependent concentration.
IHC-P	1:20,000
IHC-Fr	1:50

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

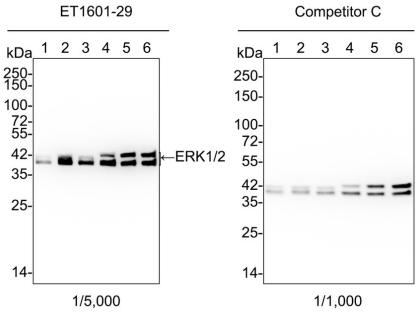


Fig1: Western blot analysis of ERK1/2 on different lysates with Rabbit anti-ERK1/2 antibody (ET1601-29) at 1/5,000 dilution and competitor's antibody at 1/1,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: Jurkat cell lysate
Lane 3: HepG2 cell lysate
Lane 4: NIH/3T3 cell lysate
Lane 5: PC-12 cell lysate
Lane 6: C6 cell lysate

Lysates/proteins at 15 µg/Lane.

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

Exposure time: 19 seconds;

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 (ET1601-29) at 1/5,000 dilution and competitor's antibody at 1/1,000 dilution were used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:50,000 dilution was used for 1 hour at room temperature.

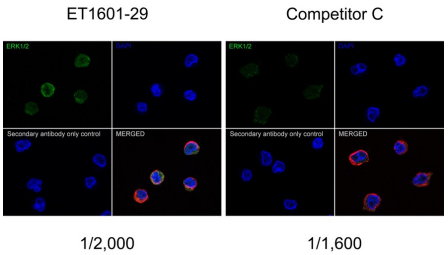


Fig2: Immunocytochemistry analysis of Jurkat cells labeling ERK1/2 with Rabbit anti-ERK1/2 antibody (ET1601-29) at 1/2,000 dilution and competitor's antibody at 1/1,600 dilution.

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-ERK1/2 antibody (ET1601-29) at 1/2,000 dilution and competitor's antibody at 1/1,600 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.

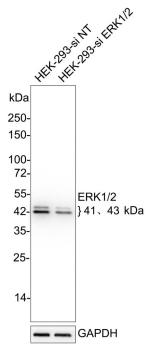


Fig3: Western blot analysis of ERK1/2 on different lysates with Rabbit anti-ERK1/2 antibody (ET1601-29) at 1/2,000 dilution.

Lane 1: HEK-293-si NT cell lysate
Lane 2: HEK-293-si ERK1/2 cell lysate

Lysates/proteins at 10 µg/Lane.

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

Exposure time: 20 seconds;

4-20% SDS-PAGE gel.

ET1601-29 was shown to specifically react with ERK1/2 in HEK-293-si NT cells. Weakened band was observed when HEK-293-si ERK1/2 sample was tested. Hela-si NT and HEK-293-si ERK1/2 samples were subjected to SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary antibody (ET1601-29, 1/2,000) and Loading control antibody (Rabbit anti-GAPDH, ET1601-4, 1/10,000) were used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-rabbit IgG-HRP Secondary Antibody (HA1001) at 1:50,000 dilution was used for 1 hour at room temperature.

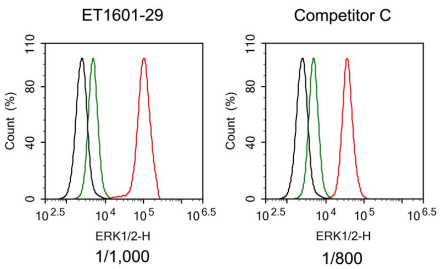


Fig4: Flow cytometric analysis of Jurkat cells labeling ERK1/2.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1601-29, red) at 1/1,000 dilution and competitor's antibody (red) at 1/800 dilution, 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).

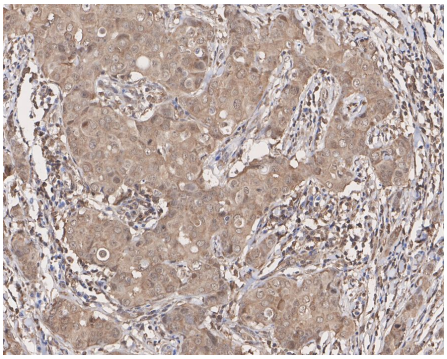


Fig5: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue with Rabbit anti-ERK1/2 antibody (ET1601-29) at 1/20,000 dilution.

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 (ET1601-29) at 1/20,000 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.

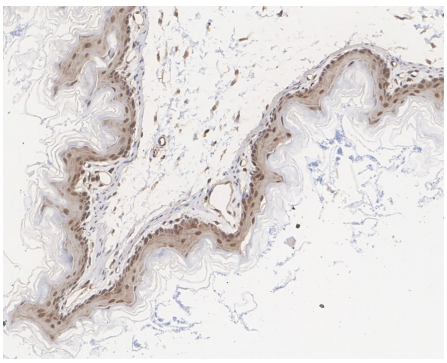


Fig6: Immunohistochemical analysis of paraffin-embedded mouse esophagus tissue with Rabbit anti-ERK1/2 antibody (ET1601-29) at 1/20,000 dilution.

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 (ET1601-29) at 1/20,000 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.

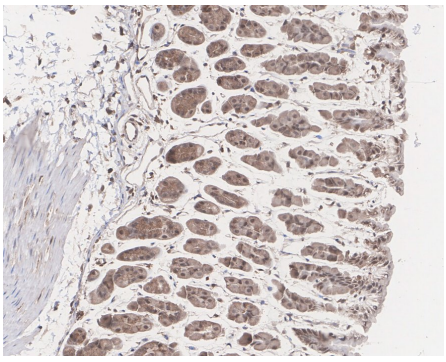


Fig7: Immunohistochemical analysis of paraffin-embedded mouse stomach tissue with Rabbit anti-ERK1/2 antibody (ET1601-29) at 1/20,000 dilution.

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 (ET1601-29) at 1/20,000 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.

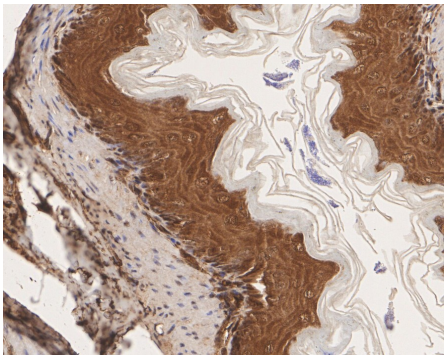


Fig8: Immunohistochemical analysis of paraffin-embedded rat esophagus tissue with Rabbit anti-ERK1/2 antibody (ET1601-29) at 1/1,000 dilution.

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 (ET1601-29) 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.

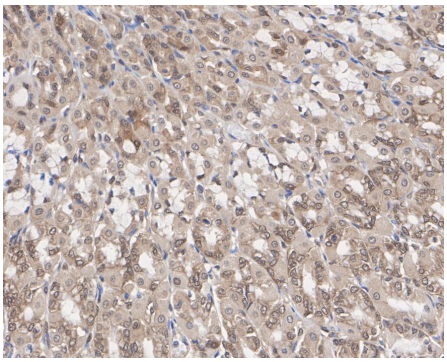


Fig9: Immunohistochemical analysis of paraffin-embedded rat stomach tissue with Rabbit anti-ERK1/2 antibody (ET1601-29) at 1/3,000 dilution.

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 (ET1601-29) at 1/3,000 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.

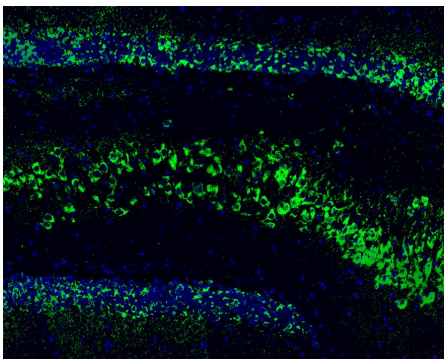


Fig10: Immunofluorescence analysis of frozen mouse hippocampus tissue labeling ERK1/2 with Rabbit anti-ERK1/2 antibody (ET1601-29).
The tissues were blocked in 3% BSA for 30 minutes at room temperature, washed with PBS, and then probed with the primary antibody (ET1601-29, green) at 1/50 dilution overnight at 4℃, washed with PBS. Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) was used as the secondary antibody at 1/200 dilution. Nuclei were counterstained with DAPI (blue). Image acquisition was performed with KFBIO KF-FL-400 Scanner.

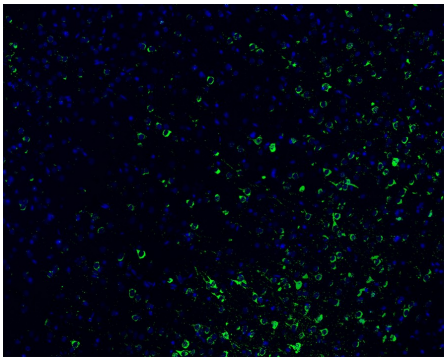


Fig11: Immunofluorescence analysis of frozen mouse cerebral cortex tissue labeling ERK1/2 with Rabbit anti-ERK1/2 antibody (ET1601-29).
The tissues were blocked in 3% BSA for 30 minutes at room temperature, washed with PBS, and then probed with the primary antibody (ET1601-29, green) at 1/50 dilution overnight at 4℃, washed with PBS. Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) was used as the secondary antibody at 1/200 dilution. Nuclei were counterstained with DAPI (blue). Image acquisition was performed with KFBIO KF-FL-400 Scanner.

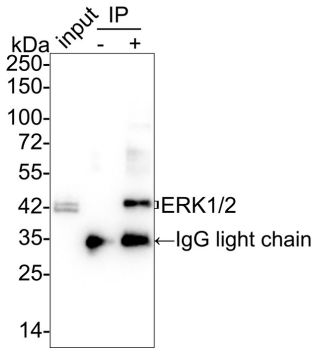
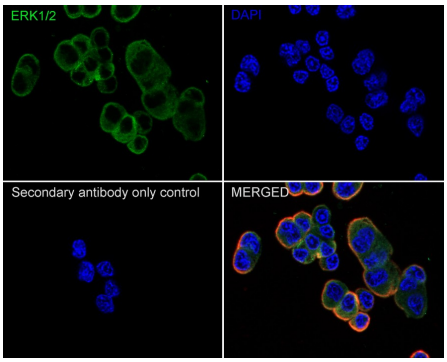


Fig12: ERK1/2 was immunoprecipitated in 0.2mg Jurkat cell lysate with ET1601-29 at 2 µg/25 µl agarose. Western blot was performed from the immunoprecipitate using ET1601-29 at 1/5,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: Jurkat cell lysate (input)
Lane 2: Rabbit IgG instead of ET1601-29 in Jurkat cell lysate
Lane 3: ET1601-29 IP in Jurkat cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 43 seconds

Fig13: Immunocytochemistry analysis of PC-12 cells labeling ERK1/2 with Rabbit anti-ERK1/2 antibody (ET1601-29) 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-ERK1/2 antibody (ET1601-29) 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.

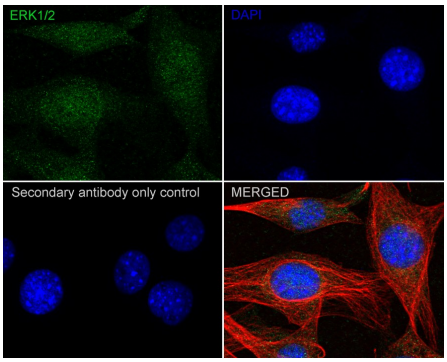


Fig14: Immunocytochemistry analysis of NIH/3T3 cells labeling ERK1/2 with Rabbit anti-ERK1/2 antibody (ET1601-29) at 1/500 dilution.

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-ERK1/2 antibody (ET1601-29) at 1/500 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.

Background References

1. Ye, Q. et al. 2014. Lactoferrin deficiency promotes colitis-associated colorectal dysplasia in mice. PloS one. 9: e103298.
2. Polidoro, L. et al. 2013. Vitamin D protects human endothelial cells from H O oxidant injury through the Mek/Erk-Sirt1 axis activation. Journal of cardiovascular translational research. 6: 221-31.