Anti-Phospho-Erk1 (T202)+Erk2 (T185) Antibody [SZ2-4]

ET1603-22

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, FC, IF-Tissue
Molecular Wt:	Predicted band size: 43/41 kDa
Clone number:	SZ2-4

Description: Mitogen-activated protein kinases (MAPKs) are a widely conserved family of serine/threonine protein kinases involved in many cellular programs, such as cell proliferation, differentiation, motility, and death. The p44/42 MAPK (Erk1/2) signaling pathway can be activated in response to a diverse range of extracellular stimuli including mitogens, growth factors, and cytokines, and research investigators consider it an important target in the diagnosis and treatment of cancer. Upon stimulation, a sequential three-part protein kinase cascade is initiated, consisting of a MAP kinase kinase kinase (MAPKKK or MAP3K), a MAP kinase kinase (MAPKK or MAP2K), and a MAP kinase (MAPK). Multiple p44/42 MAP3Ks have been identified, including members of the Raf family, as well as Mos and Tpl2/COT. MEK1 and MEK2 are the primary MAPKKs in this pathway. MEK1 and MEK2 activate p44 and p42 through phosphorylation of activation loop residues Thr202/Tyr204 and Thr185/Tyr187, respectively. Several downstream targets of p44/42 have been identified, including p90RSK and the transcription factor Elk-1. p44/42 are negatively regulated by a family of dual-specificity (Thr/Tyr) MAPK phosphatases, known as DUSPs or MKPs, along with MEK inhibitors, such as U0126 and PD98059.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Thr185 of Human Erk2.

Positive control: Jurkat treated with 200ng/mL PMA for 35 minutes cell lysate, NIH/3T3 cell lysate, NIH/3T3 treated with 200nM PMA for 30 minutes cell lysate, C6 cell lysate, C6 treated with 200nM PMA for 30 minutes cell lysate, A549, NIH/3T3, MCF-7, human lung carcinoma tissue, human kidney tissue, human gallbladder tissue.

Subcellular location: Cytoplasm, Nucleus, Membrane, Cell junction.

Database links: SwissProt: P27361 Human | P28482 Human | P63085 Mouse | Q63844 Mouse

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:100-1:500
IHC-P	1:200-1:1,000
FC	1:50-1:100
IF-Tissue	1:50-1:200

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

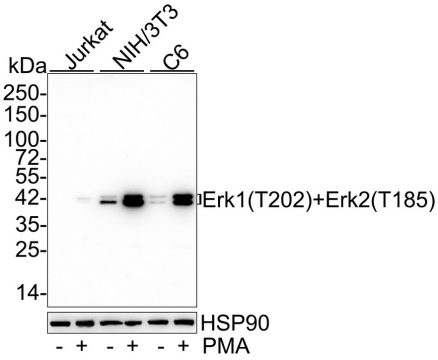

Fig1: Western blot analysis of Phospho-Erk1 (T202)+Erk2 (T185) on different lysates with Rabbit anti-Phospho-Erk1 (T202)+Erk2 (T185) antibody (ET1603-22) at 1/1,000 dilution.

Lane 1: Jurkat cell lysate
Lane 2: Jurkat treated with 200ng/mL PMA for 35 minutes cell lysate
Lane 3: NIH/3T3 cell lysate
Lane 4: NIH/3T3 treated with 200nM PMA for 30 minutes cell lysate
Lane 5: C6 cell lysate
Lane 6: C6 treated with 200nM PMA for 30 minutes cell lysate

Lysates/proteins at 10 µg/Lane.

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

Exposure time: 3 minutes;

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 (ET1603-22) at 1/1,000 dilution was 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.

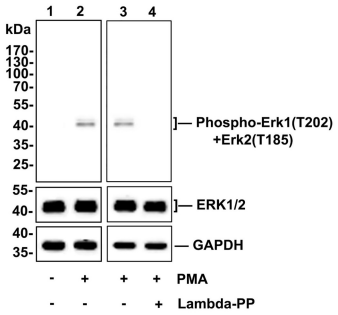

Fig2: Western blot analysis of Phospho-Erk1(T202)+Erk2(T185) on jurkat cell lysates.

Lane 1: jurkat cells, whole cell lysate, 10ug/lane
Lane 2/3: jurkat cells treated with 200 ng/ml PMA for 35 minutes, whole cell lysate, 10ug/lane
Lane 4: jurkat cells treated with 200 ng/ml PMA for 35 minutes, then treated with 2.8ug/ul lambda-PP for 30 minutes, whole cell lysates, 10ug/lane

All lanes :
Anti-Phospho-Erk1(T202)+Erk2(T185) antibody (ET1603-22) at 1/500 dilution. Anti-Erk1+Erk2antibody (ET1601-29) at 1/500 dilution. Anti-GAPDH antibody (ET1601-4) at 1/10,000 dilution. Goat Anti-Rabbit IgG H&L (HRP) (HA1001) at 1/200,000 dilution.

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

Blocking and diluting buffer: 5% BSA

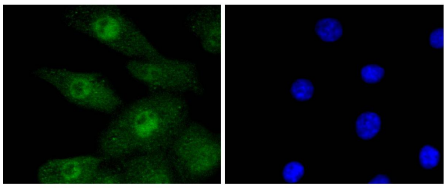

Fig3: ICC staining of Phospho-Erk1 (T202)+Erk2 (T185) in A549 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1603-22, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

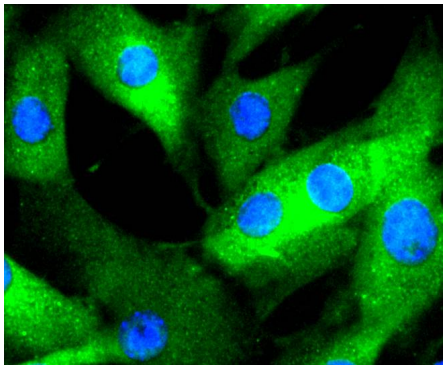

Fig4: ICC staining of Phospho-Erk1 (T202)+Erk2 (T185) in NIH/3T3 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1603-22, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

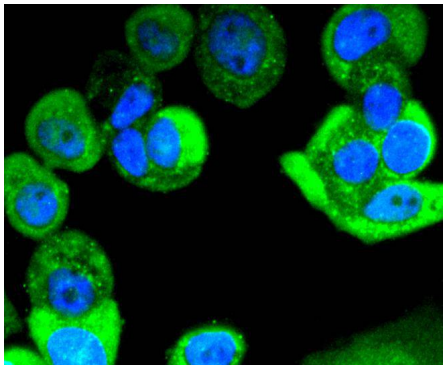

Fig5: ICC staining of Phospho-Erk1 (T202)+Erk2 (T185) in MCF-7 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1603-22, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

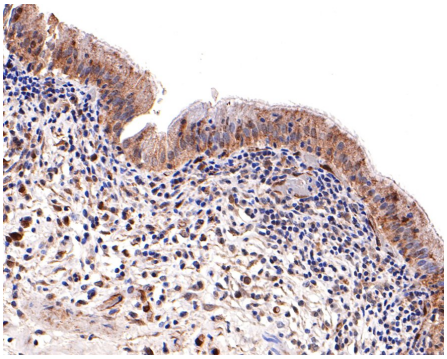

Fig6: Immunohistochemical analysis of paraffin-embedded human gallbladder tissue with Rabbit anti-Phospho-Erk1 (T202)+Erk2 (T185) antibody (ET1603-22) at 1/100 dilution.

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 (ET1603-22) at 1/100 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.

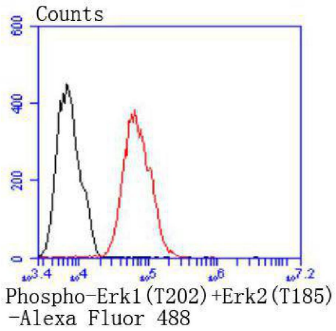

Fig7: Flow cytometric analysis of Phospho-Erk1 (T202)+Erk2 (T185) was done on MCF-7 cells. The cells were fixed, permeabilized and stained with the primary antibody (ET1603-22, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Rabbit IgG Secondary antibody at 1/1000 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

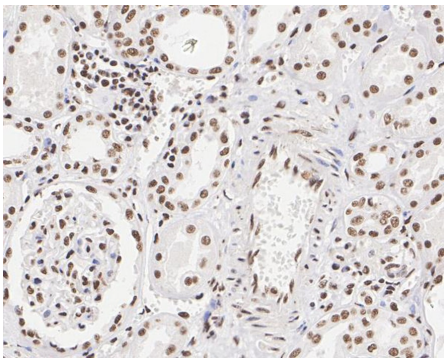

Fig8: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-Phospho-Erk1 (T202)+Erk2 (T185) antibody (ET1603-22) at 1/200 dilution.

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 (ET1603-22) at 1/200 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."

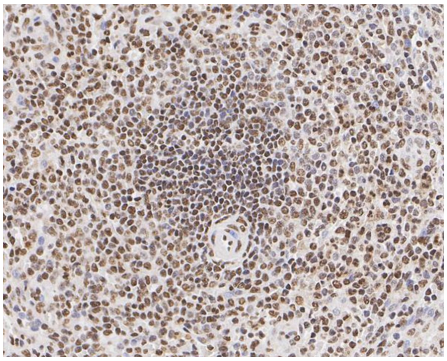

Fig9: Immunohistochemical analysis of paraffin-embedded human spleen tissue with Rabbit anti-Phospho-Erk1 (T202)+Erk2 (T185) antibody (ET1603-22) at 1/200 dilution.

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 (ET1603-22) at 1/200 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."

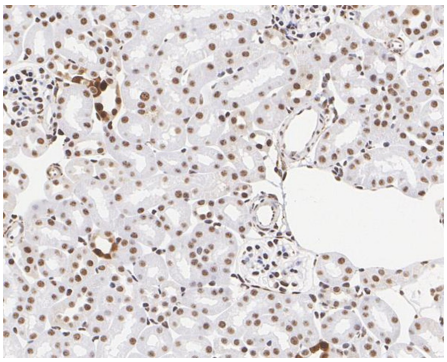

Fig10: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-Phospho-Erk1 (T202)+Erk2 (T185) antibody (ET1603-22) at 1/1,000 dilution.

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 (ET1603-22) 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."

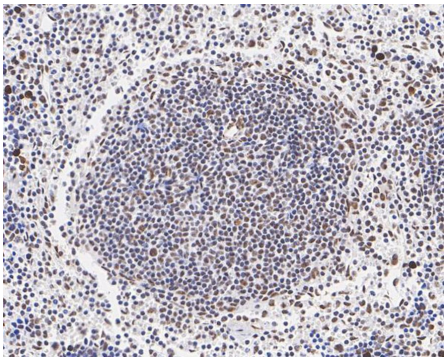

Fig11: Immunohistochemical analysis of paraffin-embedded mouse spleen tissue with Rabbit anti-Phospho-Erk1 (T202)+Erk2 (T185) antibody (ET1603-22) at 1/1,000 dilution.

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 (ET1603-22) 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."

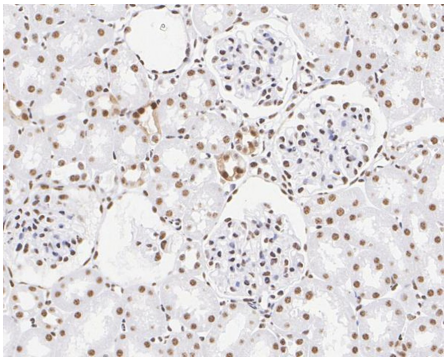

Fig12: Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Rabbit anti-Phospho-Erk1 (T202)+Erk2 (T185) antibody (ET1603-22) at 1/1,000 dilution.

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 (ET1603-22) 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."

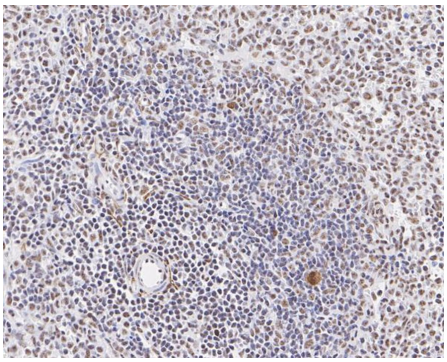

Fig13: Immunohistochemical analysis of paraffin-embedded rat spleen tissue with Rabbit anti-Phospho-Erk1 (T202)+Erk2 (T185) antibody (ET1603-22) at 1/1,000 dilution.

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 (ET1603-22) 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."

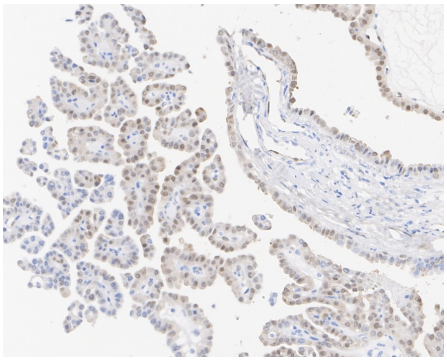

Fig14: Immunohistochemical analysis of paraffin-embedded human thyroid cancer tissue with Rabbit anti-Phospho-Erk1 (T202)+Erk2 (T185) antibody (ET1603-22) at 1/1,000 dilution.

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 (ET1603-22) 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."

Background References

1. Ruess DA et al. HDACi Valproic Acid (VPA) and Suberoylanilide Hydroxamic Acid (SAHA) Delay but Fail to Protect against Warm Hepatic Ischemia-Reperfusion Injury. PLoS One 11:e0161233 (2016).
2. Ahnstedt H et al. U0126 attenuates cerebral vasoconstriction and improves long-term neurologic outcome after stroke in female rats. J Cereb Blood Flow Metab 35:454-60 (2015).