# Anti-ERK2 Antibody [SZ25-01]

### ET1603-23



Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, IP, FC, IHC-Fr
Molecular Wt:	Predicted band size: 41 kDa
Clone number:	SZ25-01
Description:	Mitogen-activated protein kinase (MAPK) signaling pathways involve two closely related MAP kinases, known as extracellular-signal-related kinase 1 (ERK 1, p44) and 2 (ERK 2, p42). Growth factors, steroid hormones, G protein-coupled receptor ligands, and neurotransmitters can initiate MAPK signaling pathways. Activation of ERK1 and ERK2 requires phosphorylation by upstream kinases such as MAP kinase kinase (MEK), MEK kinase and Raf-1. ERK1 and ERK2 phosphorylation can occur at specific tyrosine and threonine sites mapping within consensus motifs that include the Threonine-Glutamate-Tyrosine motif. ERK activation leads to dimerization with other ERKs and subsequent localization to the nucleus. Active ERK dimers phosphorylate serine and threonine residues on nuclear proteins and influence a host of responses that include proliferation, differentiation, transcription regulation and development. The human ERK2 gene maps to chromosome 22q11.21 and encodes a 360-amino acid protein.
lmmunogen:	Synthetic peptide within Human ERK2 aa 311-360 / 360.
Positive control:	HEK-293 cell lysate, HeLa cell lysate, NIH/3T3 cell lysate, RAW264.7 cell lysate, PC-12 cell lysate, C6 cell lysate, Hela, MCF-7, NIH/3T3, human pancreas tissue, mouse pancreas tissue, rat pancreas tissue, mouse hippocampus tissue, mouse cerebral cortex tissue.
Subcellular location:	Cytoplasm, Nucleus, Cytoskeleton, Membrane, Cell junction.
Database links:	SwissProt: P28482 Human   P63085 Mouse   P63086 Rat
Recommended Dilutions:	
WB	1:2,000-1:5,000
IF-Cell	1:50-1:200
IF-Tissue	1:50-1:200
IHC-P	1:200-1:1,000
FC	1:1,000
IP IHC-Fr	Use at an assay dependent concentration. 1:100
Storage Buffer:	1*TBS (pH7.4), 0.05% BSA, 40% Glycerol, Preservative: 0.05% Sodium Azide,
Storage Instruction:	Store at +4 $^{\circ}$ after thawing. Aliquot store at -20 $^{\circ}$ or -80 $^{\circ}$ . Avoid repeated freeze / thaw cycles.
Purity:	Protein A affinity purified.

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#### Images

kDa\_50 250

ERK2

~41kDa

GAPDH

100

72 55

45

35 25

14



Lane 1: A549-si NT cell lysate Lane 2: A549-si ERK2 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 41 kDa Observed band size: 41 kDa

Exposure time: 1 minute; 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 (ET1603-23) at 1/2,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. 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 ERK2 on different lysates with Rabbit anti-ERK2 antibody (ET1603-23) at 1/2,000 dilution.

Lane 1: HEK-293 cell lysate Lane 2: HeLa cell lysate Lane 3: NIH/3T3 cell lysate Lane 4: RAW264.7 cell lysate Lane 5: PC-12 cell lysate Lane 6: C6 cell lysate

Lysates/proteins at 15 µg/Lane.

Predicted band size: 41 kDa Observed band size: 41 kDa

Exposure time: 3 minutes; 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 (ET1603-23) at 1/2,000 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.

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ERK2

HSP90

41kDa

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kDa 250 150

55

35 25

14

Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation

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**Fig3:** ICC staining of ERK2 in Hela 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 (ET1603-23, 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 ERK2 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 (ET1603-23, 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 ERK2 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 (ET1603-23, 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 pancreas tissue with Rabbit anti-ERK2 antibody (ET1603-23) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1603-23) 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.

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**Fig7:** Immunohistochemical analysis of paraffin-embedded mouse pancreas tissue with Rabbit anti-ERK2 antibody (ET1603-23) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1603-23) 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.





**Fig8:** Immunohistochemical analysis of paraffin-embedded rat pancreas tissue with Rabbit anti-ERK2 antibody (ET1603-23) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1603-23) 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:** Immunofluorescence analysis of frozen mouse hippocampus tissue labeling ERK2 with Rabbit anti-ERK2 antibody (ET1603-23).

The tissues were blocked in 3% BSA for 30 minutes at room temperature, washed with PBS, and then probed with the primary antibody ((ET1603-23, green) at 1/100 dilution overnight at 4°C, 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.

**Fig10:** Immunofluorescence analysis of frozen mouse cerebral cortex tissue labeling ERK2 with Rabbit anti-ERK2 antibody (ET1603-23).

The tissues were blocked in 3% BSA for 30 minutes at room temperature, washed with PBS, and then probed with the primary antibody ((ET1603-23, green) at 1/100 dilution overnight at 4 $^{\circ}$ C, 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.

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Fig11: Flow cytometric analysis of NIH/3T3 cells labeling ERK2.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1603-23, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4°C for an hour, the cells were stained with a iFluor<sup>TM</sup> 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4°C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

**Fig12:** Immunofluorescence analysis of paraffin-embedded mouse pancreas tissue labeling ERK2 with Rabbit anti-ERK2 antibody (ET1603-23) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1603-23, green) at 1/200 dilution overnight at 4 °C, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

**Fig13:** Immunofluorescence analysis of paraffin-embedded rat brain tissue labeling ERK2 with Rabbit anti-ERK2 antibody (ET1603-23) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1603-23, green) at 1/200 dilution overnight at 4 °C, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

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

#### **Background References**

- 1. Chen M et al. Targeting primitive chronic myeloid leukemia cells by effective inhibition of a new AHI-1-BCR-ABL-JAK2 complex. J Natl Cancer Inst 105:405-23 (2013).
- 2. Emmanuel C et al. Comparison of expression profiles in ovarian epithelium in vivo and ovarian cancer identifies novel candidate genes involved in disease pathogenesis. PLoS One 6:e17617 (2011).

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