Anti-MEK1 Antibody [SZ22-01]

ET1603-20

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Cow, Dog
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, IP, FC
Molecular Wt:	Predicted band size: 43 kDa
Clone number:	SZ22-01

Description: Dual specificity protein kinase which acts as an essential component of the MAP kinase signal transduction pathway. Binding of extracellular ligands such as growth factors, cytokines and hormones to their cell-surface receptors activates RAS and this initiates RAF1 activation. RAF1 then further activates the dual-specificity protein kinases MAP2K1/MEK1 and MAP2K2/MEK2. Both MAP2K1/MEK1 and MAP2K2/MEK2 function specifically in the MAPK/ERK cascade, and catalyze the concomitant phosphorylation of a threonine and a tyrosine residue in a Thr-Glu-Tyr sequence located in the extracellular signal-regulated kinases MAPK3/ERK1 and MAPK1/ERK2, leading to their activation and further transduction of the signal within the MAPK/ERK cascade. Activates BRAF in a KSR1 or KSR2-dependent manner; by binding to KSR1 or KSR2 releases the inhibitory intramolecular interaction between KSR1 or KSR2 protein kinase and N-terminal domains which promotes KSR1 or KSR2-BRAF dimerization and BRAF activation.

Immunogen: Synthetic peptide within N-terminal human MEK1.

Positive control: HCT 116 cell lysate, HeLa cell lysate, HepG2 cell lysate, NIH/3T3 cell lysate, Mouse brain tissue lysate, Rat skeletal muscle tissue lysate, HeLa, NIH/3T3, human tonsil tissue, mouse pancreas tissue, rat pancreas tissue.

Subcellular location: Cytoplasm, Nucleus, Membrane, Cytoskeleton.

Database links: SwissProt: Q02750 Human | P31938 Mouse | Q01986 Rat

Recommended Dilutions:

WB	1:1,000-1:2,000
IF-Cell	1:50-1:200
IF-Tissue	1:50-1:200
IHC-P	1:50-1:200
FC	1:100
IP	1-2μg/sample

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

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

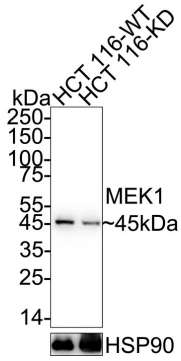

Fig1: Western blot analysis of MEK1 on different lysates with Rabbit anti-MEK1 antibody (ET1603-20) at 1/2,000 dilution.

Lane 1: HCT 116-si NT cell lysate
Lane 2: HCT 116-si MEK1 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 43 kDa
Observed band size: 45 kDa

Exposure time: 8 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 (ET1603-20) 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.

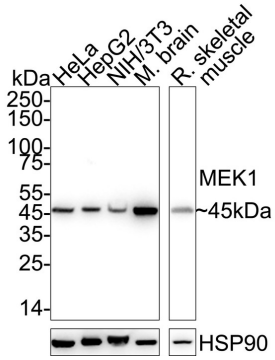

Fig2: Western blot analysis of MEK1 on different lysates with Rabbit anti-MEK1 antibody (ET1603-20) at 1/1,000 dilution.

Lane 1: HeLa cell lysate (20 µg/Lane)
Lane 2: HepG2 cell lysate (20 µg/Lane)
Lane 3: NIH/3T3 cell lysate (20 µg/Lane)
Lane 4: Mouse brain tissue lysate (30 µg/Lane)
Lane 5: Rat skeletal muscle tissue lysate (30 µg/Lane)

Predicted band size: 43 kDa
Observed band size: 45 kDa

Exposure time: 12 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 (ET1603-20) 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.

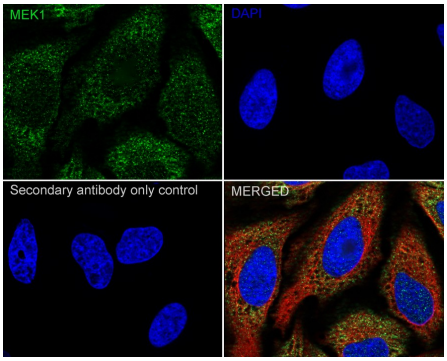

Fig3: Immunocytochemistry analysis of HeLa cells labeling MEK1 with Rabbit anti-MEK1 antibody (ET1603-20) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 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-MEK1 antibody (ET1603-20) 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 (HA601187, 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.

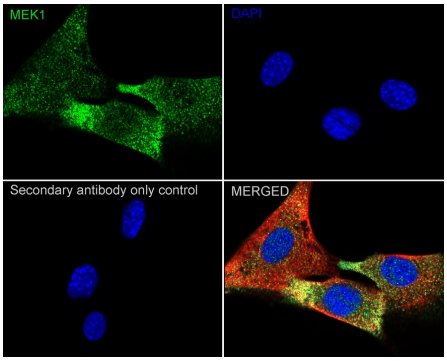

Fig4: Immunocytochemistry analysis of NIH/3T3 cells labeling MEK1 with Rabbit anti-MEK1 antibody (ET1603-20) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 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-MEK1 antibody (ET1603-20) 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 (HA601187, 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.

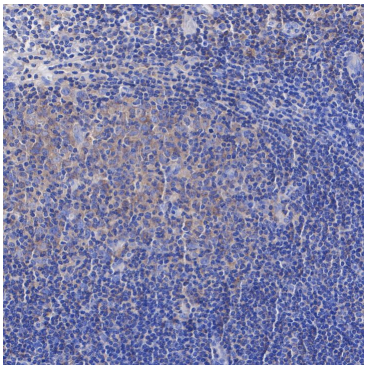

Fig5: Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Rabbit anti-MEK1 antibody (ET1603-20) at 1/50 dilution.

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_2O and PBS, and then probed with the primary antibody (ET1603-20) at 1/50 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.

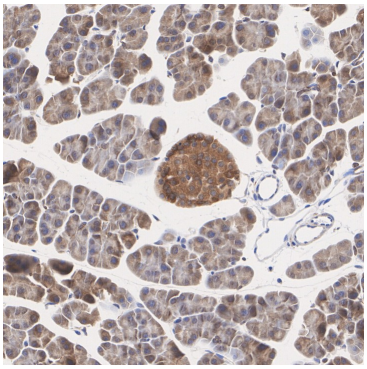

Fig6: Immunohistochemical analysis of paraffin-embedded mouse pancreas tissue with Rabbit anti-MEK1 antibody (ET1603-20) at 1/200 dilution.

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_2O and PBS, and then probed with the primary antibody (ET1603-20) 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.

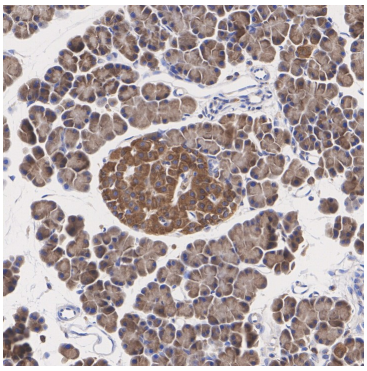

Fig7: Immunohistochemical analysis of paraffin-embedded rat pancreas tissue with Rabbit anti-MEK1 antibody (ET1603-20) at 1/200 dilution.

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_2O and PBS, and then probed with the primary antibody (ET1603-20) 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.

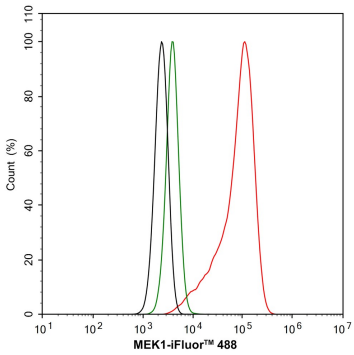

Fig8: Flow cytometric analysis of HeLa cells labeling MEK1.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1603-20, 1/100) (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).

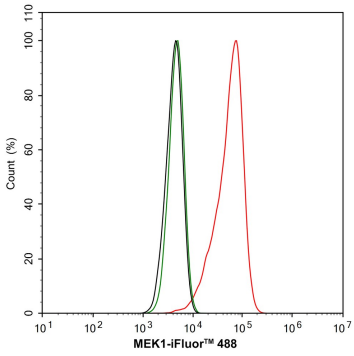

Fig9: Flow cytometric analysis of NIH/3T3 cells labeling MEK1.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1603-20, 1/100) (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).

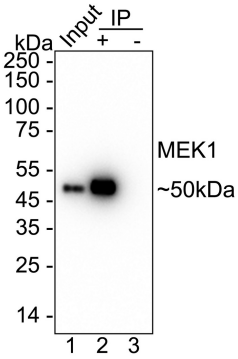

Fig10: MEK1 was immunoprecipitated from 0.2 mg HeLa cell lysate with ET1603-20 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using ET1603-20 at 1/1,000 dilution. HRP Conjugated Anti-Rabbit IgG for IP Nano-secondary antibody at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: HeLa cell lysate (input)
Lane 2: ET1603-20 IP in HeLa cell lysate
Lane 3: Rabbit IgG instead of ET1603-20 in HeLa cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 3 seconds; ECL: K1801

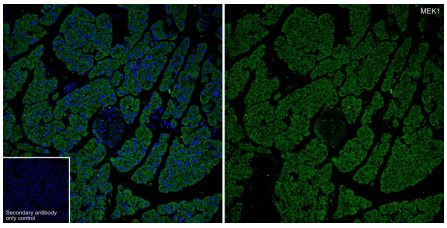

Fig11: Application: IF-Tissue

Species: Mouse

Site: pancreas

Sample: Paraffin-embedded section

Antibody concentration: 1/200

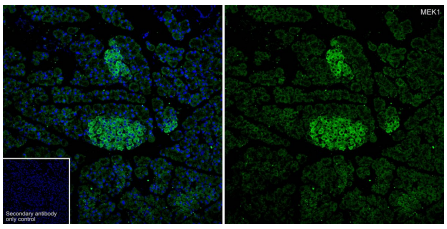

Fig12: Application: IF-Tissue

Species: Rat

Site: pancreas

Sample: Paraffin-embedded section

Antibody concentration: 1/200

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

Background References

1. Chou YY et al. Colocalization of different influenza viral RNA segments in the cytoplasm before viral budding as shown by single-molecule sensitivity FISH analysis. PLoS Pathog 9:e1003358 (2013).
2. Nath S et al. MUC1 induces drug resistance in pancreatic cancer cells via upregulation of multidrug resistance genes. Oncogenesis 2:e51 (2013).