Anti-Phospho-BRAF (T401) Antibody [JJ08-72] ET1701-20

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IP, IHC-P
Molecular Wt:	Predicted band size: 84 kDa
Clone number:	JJ08-72

Description: BRAF is a human gene that encodes a protein called B-Raf. The gene is also referred to as proto-oncogene B-Raf and v-Raf murine sarcoma viral oncogene homolog B, while the protein is more formally known as serine/threonine-protein kinase B-Raf. B-Raf is a member of the Raf kinase family of growth signal transduction protein kinases. This protein plays a role in regulating the MAP kinase/ERKs signaling pathway, which affects cell division, differentiation, and secretion. The B-Raf protein is involved in sending signals inside cells which are involved in directing cell growth. Certain other inherited BRAF mutations cause birth defects. Drugs that treat cancers driven by BRAF mutations have been developed. Two of these drugs, vemurafenib and dabrafenib are approved by FDA for treatment of late-stage melanoma.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Thr401 of Human BRAF aa 371-420 / 766.

Positive control: Mouse testis tissue lysates, PC-12 cell lysates, PC-3M, MCF-7, PC-12, human testis tissue, mouse testis tissue, rat testis tissue, mouse brain tissue, rat brain tissue.

Subcellular location: Nucleus, Cytoplasm, Cell membrane.

Database links: SwissProt: P15056 Human | P28028 Mouse
Entrez Gene: 114486 Rat

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:100
IP	Use at an assay dependent concentration.
IHC-P	1:200

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

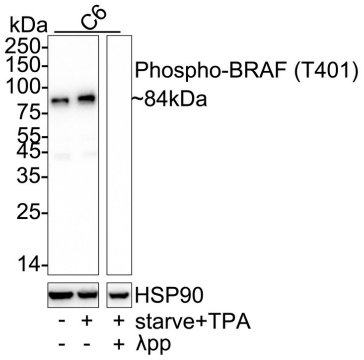

Fig1: Western blot analysis of Phospho-BRAF (T401) on different lysates with Rabbit anti-Phospho-BRAF (T401) antibody (ET1701-20) at 1/2,000 dilution.

Lane 1: C6 cell lysate
Lane 2: C6 starved overnight then treated with 200nM TPA for 4 hours cell lysate
Lane 3: C6 starved overnight then treated with 200nM TPA for 4 hours cell lysate, then the membrane treated with λpp for 1 hour

Lysates/proteins at 20 µg/Lane.

Predicted band size: 84 kDa
Observed band size: 84 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 (ET1701-20) 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.

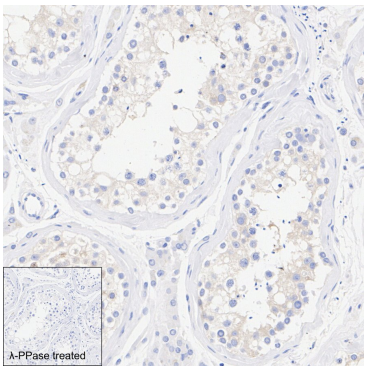

Fig2: Immunohistochemical analysis of paraffin-embedded human testis tissue untreated / treated with λpp with Rabbit anti-Phospho-BRAF (T401) antibody (ET1701-20) at 1/200 dilution.

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 (ET1701-20) 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.

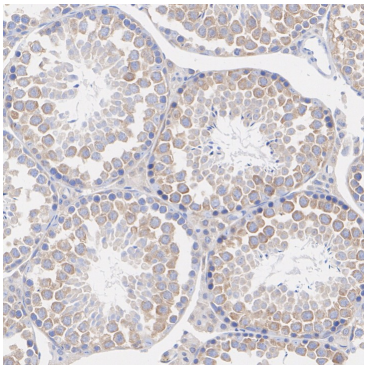

Fig3: Immunohistochemical analysis of paraffin-embedded mouse testis tissue with Rabbit anti-Phospho-BRAF (T401) antibody (ET1701-20) at 1/200 dilution.

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 (ET1701-20) 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.

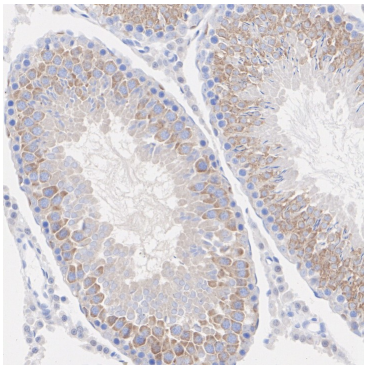

Fig4: Immunohistochemical analysis of paraffin-embedded rat testis tissue with Rabbit anti-Phospho-BRAF (T401) antibody (ET1701-20) at 1/200 dilution.

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 (ET1701-20) 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.

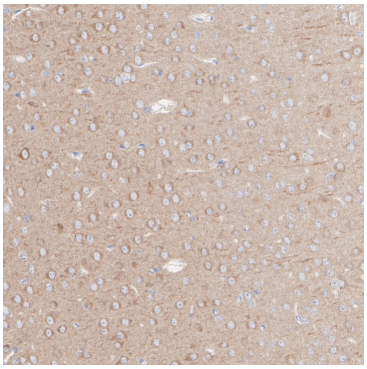

Fig5: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-Phospho-BRAF (T401) antibody (ET1701-20) at 1/200 dilution.

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 (ET1701-20) 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.

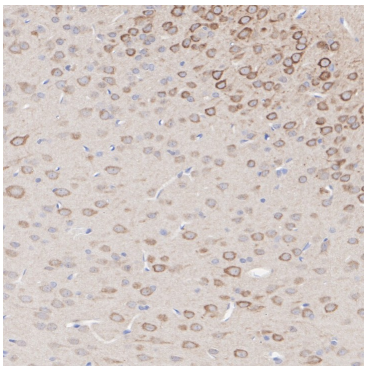

Fig6: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-Phospho-BRAF (T401) antibody (ET1701-20) at 1/200 dilution.

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 (ET1701-20) 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.

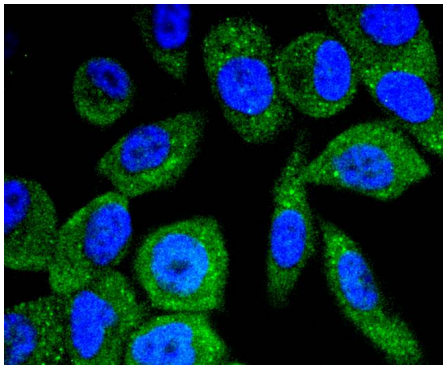

Fig7: ICC staining of Phospho-BRAF (T401) in PC-3M cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 10% negative goat serum for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1701-20, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

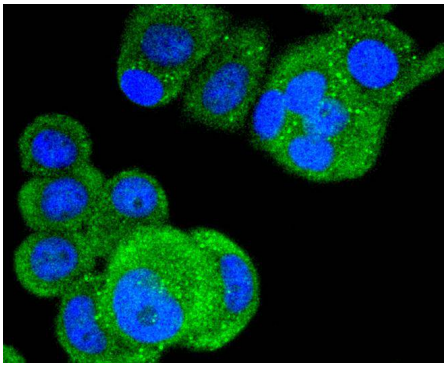

Fig8: ICC staining of Phospho-BRAF (T401) in MCF-7 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 10% negative goat serum for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1701-20, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

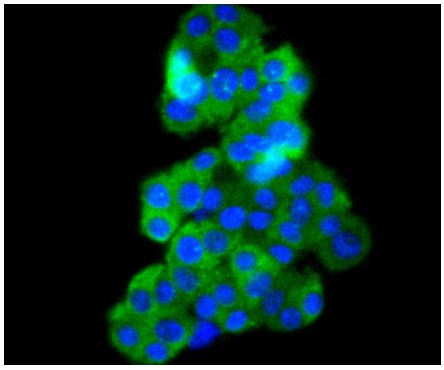

Fig9: ICC staining of Phospho-BRAF (T401) in PC-12 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 10% negative goat serum for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1701-20, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

Background References

1. Brown WS et al. B-Raf regulation of integrin a4 1-mediated resistance to shear stress through changes in cell spreading and cytoskeletal association in T cells. J Biol Chem 289:23141-53 (2014).
2. Wada, M. et al. The dual RAF/MEK inhibitor CH5126766/RO5126766 may be a potential therapy for RAS-mutated tumor cells. PloS one. 9: e113217(2014).