Anti-BRAF Antibody [F1-F4-E10]

EM1701-32

Product Type:	Mouse monoclonal IgG2b, primary antibodies
Species reactivity:	Human, Mouse, Rat, Monkey
Applications:	WB, IHC-P, IF-Cell, FC
Molecular Wt:	Predicted band size: 84 kDa
Clone number:	F1-F4-E10

Description: Several serine/threonine protein kinases have been implicated as intermediates in signal transduction pathways. These include ERK/MAP kinases, ribosomal S6 kinase (Rsk) and Raf-1. Raf-1 is a cytoplasmic protein with intrinsic serine/threonine activity. It is broadly expressed in nearly all cell lines tested to date and is the cellular homolog of v-Raf, the product of the transforming gene of the 3611 strain of murine sarcoma virus. The unregulated kinase activity of the v-Raf protein has been associated with transformation and mitogenesis, while the activity of Raf-1 is normally suppressed by a regulatory N-terminal domain. Raf-A, a second member of the Raf gene family of serine/threonine protein kinases, exhibits substantial homology to Raf-1 within the kinase domain of the two molecules, but less homology elsewhere. Expression of Raf-B is highly restricted, with highest levels in the cerebrum and testis.

Immunogen: Recombinant protein within Human BRAF aa 49-239 / 766.

Positive control: HeLa cell lysate, K-562 cell lysate, HT-29 cell lysate, SK-MEL-28 cell lysate, HEK-293 cell lysate, SiHa cell lysate, COS-1 cell lysate, Vero cell lysate, PC-12 cell lysate, HAP1 cell lysate, rat testis tissue, human testis tissue, human colon cancer tissue, K-562.

Subcellular location: Nucleus, Cytoplasm, Cell membrane.

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

Recommended Dilutions:

WB	1:500-1:2,000
IF-Cell	1:100
IHC-P	1:500-1:1,000
FC	1:1,000

Storage Buffer: 1*PBS (pH7.4), 0.2% BSA, 50% 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

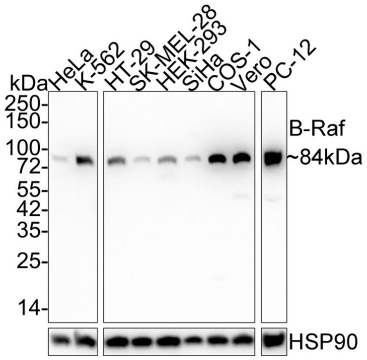

Fig1: Western blot analysis of BRAF on different lysates with Mouse anti-BRAF antibody (EM1701-32) at 1/2,000 dilution.

Lane 1: HeLa cell lysate (20 µg/Lane)
Lane 2: K-562 cell lysate (20 µg/Lane)
Lane 3: HT-29 cell lysate (20 µg/Lane)
Lane 4: SK-MEL-28 cell lysate (20 µg/Lane)
Lane 5: HEK-293 cell lysate (20 µg/Lane)
Lane 6: SiHa cell lysate (20 µg/Lane)
Lane 7: COS-1 cell lysate (20 µg/Lane)
Lane 8: Vero cell lysate (20 µg/Lane)
Lane 9: PC-12 cell lysate (20 µg/Lane)

Predicted band size: 84 kDa
Observed band size: 84 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 (EM1701-32) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

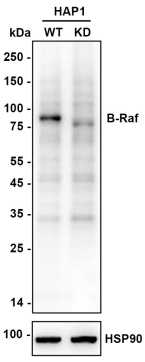

Fig2: Western blot analysis of BRAF on different lysates with Mouse anti-BRAF antibody (EM1701-32) at 1/5,000 dilution.

Lane 1: HAP1-parental cell lysate (10 µg/Lane)
Lane 2: HAP1-BRAF KD cell lysate (10 µg/Lane)

Predicted band size: 84 kDa
Observed band size: 84 kDa

Exposure time: 30 seconds; ECL: K1802;
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 (EM1701-32) at 1/2,000 dilution was used in K1803 at 4℃ overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

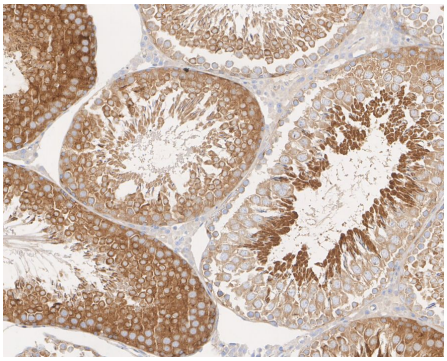

Fig3: Immunohistochemical analysis of paraffin-embedded rat testis tissue with Mouse anti-BRAF antibody (EM1701-32) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) 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 (EM1701-32) at 1/2,000 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.

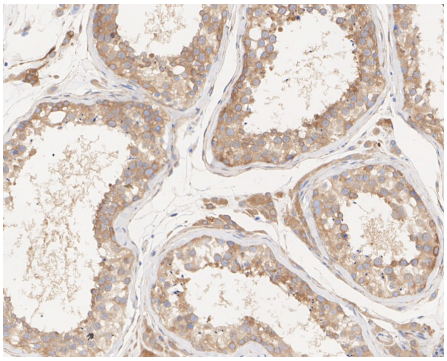

Fig4: Immunohistochemical analysis of paraffin-embedded human testis tissue with Mouse anti-BRAF antibody (EM1701-32) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) 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 (EM1701-32) at 1/2,000 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.

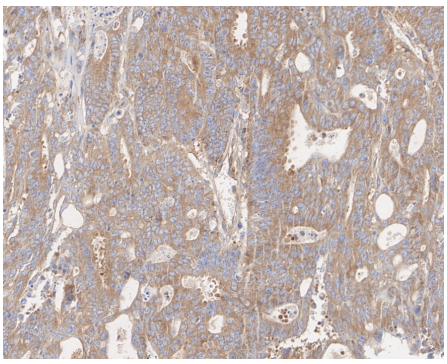

Fig5: Immunohistochemical analysis of paraffin-embedded human colon cancer tissue with Mouse anti-BRAF antibody (EM1701-32) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) 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 (EM1701-32) at 1/500 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.

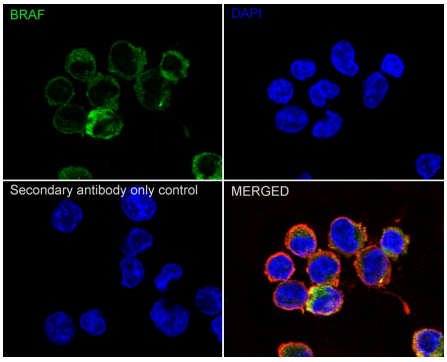

Fig6: Immunocytochemistry analysis of K-562 cells labeling BRAF with Mouse anti-BRAF antibody (EM1701-32) at 1/100 dilution.

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 Mouse anti-BRAF antibody (EM1701-32) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Mouse IgG H&L (iFluor™ 488, HA1125) 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 (ET1602-4, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Rabbit IgG H&L (iFluor™ 594, HA1122) were used as the secondary antibody at 1/1,000 dilution.

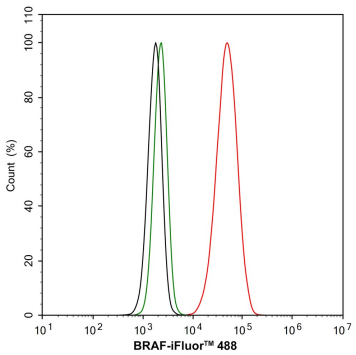

Fig7: Flow cytometric analysis of K-562 cells labeling BRAF.

Cells were fixed and permeabilized. Then stained with the primary antibody (EM1701-32, 1/1,000) (red) compared with Mouse 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-Mouse IgG Secondary antibody (HA1125) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

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

Background References

1. van Veen JE et al. P2A-Fluorophore Tagging of BRAF Tightly Links Expression to Fluorescence In Vivo. PLoS One 11:e0157661 (2016).
2. Ding Z et al. Griffipavixanthone, a dimeric xanthone extracted from edible plants, inhibits tumor metastasis and proliferation via downregulation of the RAF pathway in esophageal cancer. Oncotarget 7:1826-37 (2016).