Anti-53BP1 Antibody [JA66-11]

ET1704-05

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, FC
Molecular Wt:	Predicted band size: 214 kDa
Clone number:	JA66-11

Description: The p53 binding proteins 53BP1 and 53BP2 (Bbp) bind to the central DNA-binding domain of wild type p53, but do not bind mutant p53. The central DNA-binding domain of p53 is required for site-specific DNA binding and is frequently mutated in malignant tumors. Binding of 53BP1 to the L3 loop of p53 and of 53BP2 to the L2 loop of p53 confirms that the loop is dependent on p53 conformation. Site-specific binding also suggests that 53BP1 and 53BP2 are involved in p53-mediated tumor suppression. 53BP1 was isolated from H258 cells and is expressed in Jurkat cells in both the cytoplasm and the nucleus. The N-terminus of 53BP2 is localized to the cytoplasm, while the C-terminus might be localized in the nucleus. 53BP1 promotes cell proliferation by binding to p202, whereas 53BP2 induces cell death by binding to Bcl2 and NFkB p65.

Immunogen: Synthetic peptide within Human 53BP1 aa 1-50 / 1972.

Positive control: HeLa cell lysate, HT-29 cell lysate, human brain tissue lysate, mouse brain tissue lysate, rat heart tissue lysate, HeLa, human brain tissue, mouse spleen tissue, mouse testis tissue, rat testis tissue, human spleen tissue, human liver tissue, human cervix tissue.

Subcellular location: Nucleus. Chromosome.

Database links: SwissProt: Q12888 Human | P70399 Mouse
Entrez Gene: 296099 Rat

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:50
IF-Tissue	1:200
IHC-P	1:200-1:5,000
FC	1:1,000

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

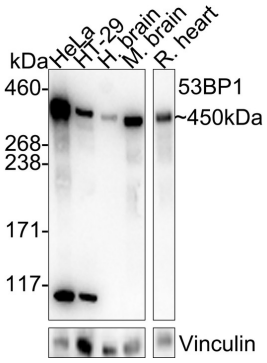

Fig1: Western blot analysis of 53BP1 on different lysates with Rabbit anti-53BP1 antibody (ET1704-05) at 1/2,000 dilution.

Lane 1: HeLa cell lysate (15 µg/Lane)
Lane 2: HT-29 cell lysate (15 µg/Lane)
Lane 3: Human brain tissue lysate (20 µg/Lane)
Lane 4: Mouse brain tissue lysate (20 µg/Lane)
Lane 5: Rat heart tissue lysate (20 µg/Lane)

Predicted band size: 214 kDa
Observed band size: 450 kDa

Exposure time: 43 seconds; ECL: K1801;

3-8% 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 (ET1704-05) 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.

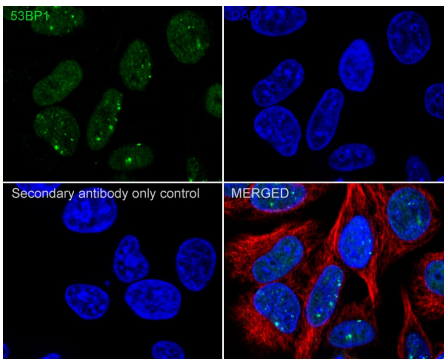

Fig2: Immunocytochemistry analysis of HeLa cells labeling 53BP1 with Rabbit anti-53BP1 antibody (ET1704-05) at 1/50 dilution.

Cells were fixed in 100% precooled methanol for 5 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-53BP1 antibody (ET1704-05) at 1/50 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 (M1305-2, 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.

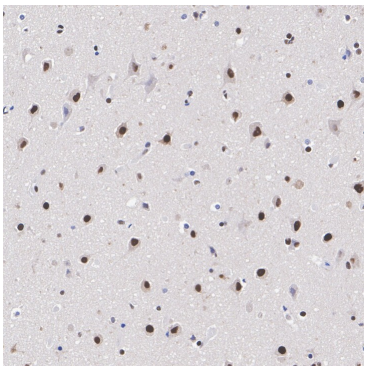

Fig3: Immunohistochemical analysis of paraffin-embedded human brain tissue with Rabbit anti-53BP1 antibody (ET1704-05) at 1/200 dilution.

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 (ET1704-05) 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.

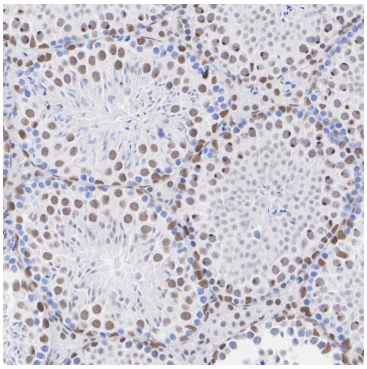

Fig4: Immunohistochemical analysis of paraffin-embedded mouse testis tissue with Rabbit anti-53BP1 antibody (ET1704-05) at 1/5,000 dilution.

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_2O and PBS, and then probed with the primary antibody (ET1704-05) at 1/5,000 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.

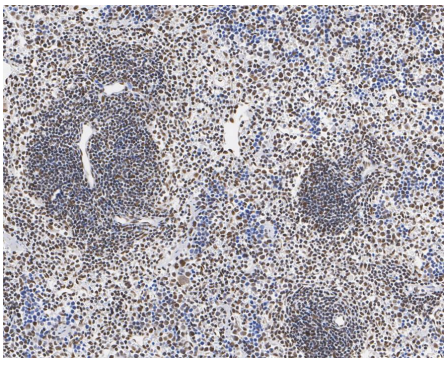

Fig5: Immunohistochemical analysis of paraffin-embedded mouse spleen tissue with Rabbit anti-53BP1 antibody (ET1704-05) at 1/200 dilution.

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 (ET1704-05) 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.

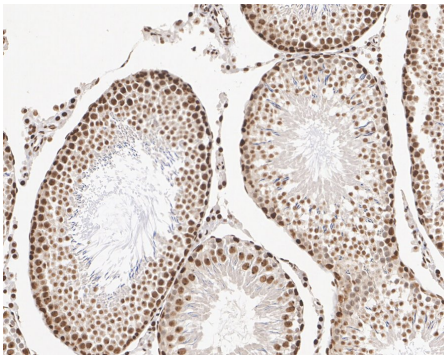

Fig6: Immunohistochemical analysis of paraffin-embedded rat testis tissue with Rabbit anti-53BP1 antibody (ET1704-05) at 1/200 dilution.

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 (ET1704-05) 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.

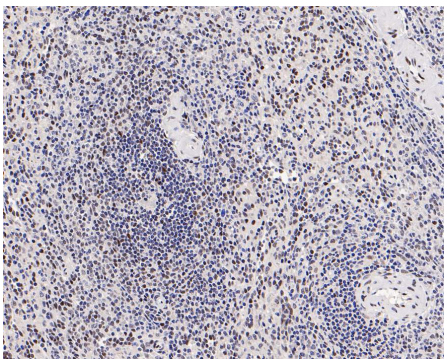

Fig7: Immunohistochemical analysis of paraffin-embedded human spleen tissue with Rabbit anti-53BP1 antibody (ET1704-05) at 1/200 dilution.

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 (ET1704-05) 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.

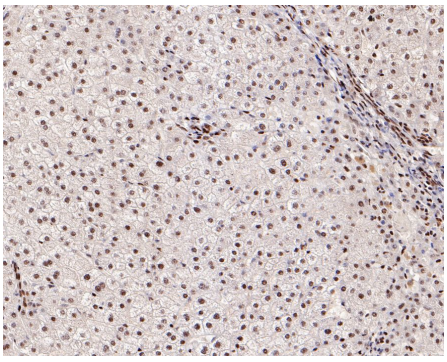

Fig8: Immunohistochemical analysis of paraffin-embedded human liver tissue with Rabbit anti-53BP1 antibody (ET1704-05) at 1/200 dilution.

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 (ET1704-05) 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.

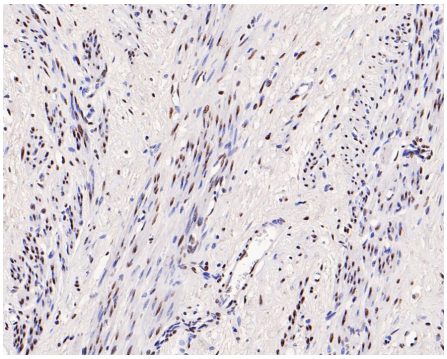

Fig9: Immunohistochemical analysis of paraffin-embedded human cervix tissue with Rabbit anti-53BP1 antibody (ET1704-05) at 1/200 dilution.

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 (ET1704-05) 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.

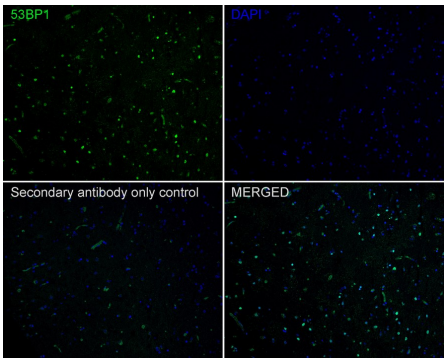

Fig10: Immunofluorescence analysis of paraffin-embedded human brain tissue labeling 53BP1 with Rabbit anti-53BP1 antibody (ET1704-05) at 1/200 dilution.

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 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1704-05, green) at 1/200 dilution overnight at 4 ℃, 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).

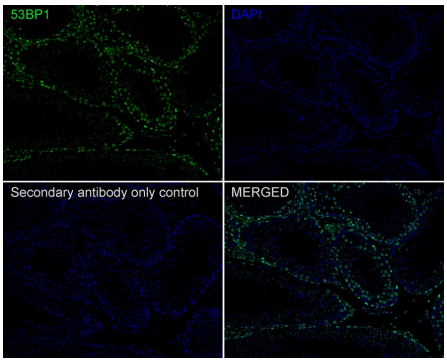

Fig11: Immunofluorescence analysis of paraffin-embedded mouse testis tissue labeling 53BP1 with Rabbit anti-53BP1 antibody (ET1704-05) at 1/200 dilution.

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 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1704-05, green) at 1/200 dilution overnight at 4 ℃, 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).

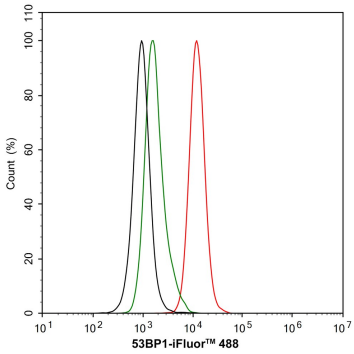

Fig12: Flow cytometric analysis of HeLa cells labeling 53BP1.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1704-05, 1/1,000) (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).

Background References

1. Manchon JF et al. Levetiracetam mitigates doxorubicin-induced DNA and synaptic damage in neurons. Sci Rep 6:25705 (2016).
2. Alessio N et al. Low dose radiation induced senescence of human mesenchymal stromal cells and impaired the autophagy process. Oncotarget 6:8155-66 (2015).