Anti-Dnmt1 Antibody [JF09-89]

ET1702-77

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P
Molecular Wt:	Predicted band size: 183 kDa
Clone number:	JF09-89

Description: Methylation at the 5'-position of cytosine is the only known naturally occurring covalent modification of the mammalian genome. DNA methylation requires the enzymatic activity of DNA 5-cytosine methyltransferase (Dnmt) proteins, which catalyze the transfer of a methyl group from S-adenosyl methionine to the 5'-position of cytosines residing in the dinucleotide CpG motif, and this methylation results in transcriptional repression of the target gene. The Dnmt enzymes are encoded by independent genes. Dnmt1 is the most abundant, and it preferentially methylates hemimethylated DNA and coordinates gene expression during development. Additional mammalian Dnmt proteins include Dnmt2 and Dnmt3. Dnmt2 lacks the large N-terminal regulator domain of Dnmt1, is expressed at substantially lower levels in adult tissues, and is likely involved in methylating newly integrated retroviral DNA. Dnmt3a and Dnmt3b are encoded by two distinct genes, but both are abundantly expressed in embryonic stem cells, where they also methylate CpG motifs on DNA.

Immunogen: Synthetic peptide within Human Dnmt1 aa 1,506-1,549 / 1,616.

Positive control: HepG2 cell lysates, Hela, HepG2, 293T, human lymph nodes tissue, F9.

Subcellular location: Nucleus.

Database links: SwissProt: P26358 Human | P13864 Mouse | Q9Z330 Rat

Recommended Dilutions:

WB	1:500-1:2,000
IF-Cell	1:50-1:200
IF-Tissue	1:50-1:200
IHC-P	1:50-1:1.000

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

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

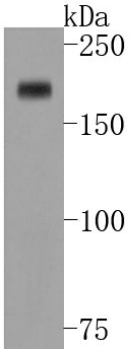

Fig1: Western blot analysis of Dnmt1 on HepG2 cell lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (ET1702-77, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:5,000 dilution was used for 1 hour at room temperature.

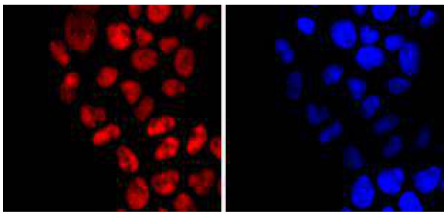

Fig2: ICC staining of Dnmt1 in Hela cells (red). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1702-77, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®594 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

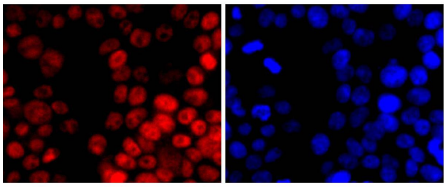

Fig3: ICC staining of Dnmt1 in 293T cells (red). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1702-77, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®594 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

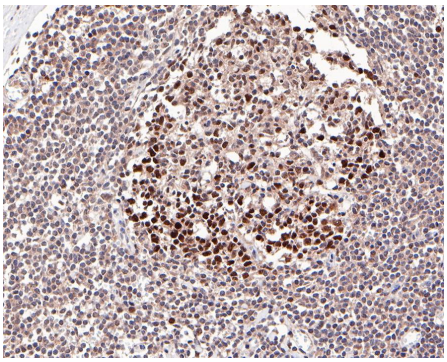

Fig4: Immunohistochemical analysis of paraffin-embedded human lymph nodes tissue with Rabbit anti-Dnmt1 antibody (ET1702-77) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) 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 (ET1702-77) 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.

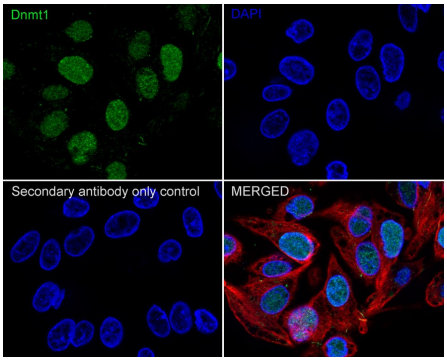

Fig5: Immunocytochemistry analysis of HepG2 cells labeling Dnmt1 with Rabbit anti-Dnmt1 antibody (ET1702-77) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS 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-Dnmt1 antibody (ET1702-77) 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 (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.

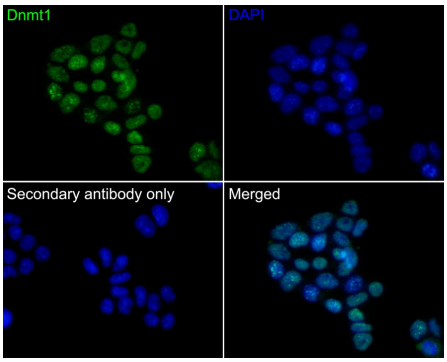

Fig6: Immunocytochemistry analysis of F9 cells labeling Dnmt1 with Rabbit anti-Dnmt1 antibody (ET1702-77) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-Dnmt1 antibody (ET1702-77) at 1/50 dilution in 2% negative goat serum 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.

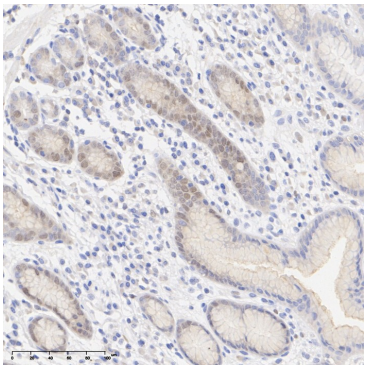

Fig7: Immunohistochemical analysis of paraffin-embedded human stomach tissue with Rabbit anti-Dnmt1 antibody (ET1702-77) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) 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 (ET1702-77) 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.

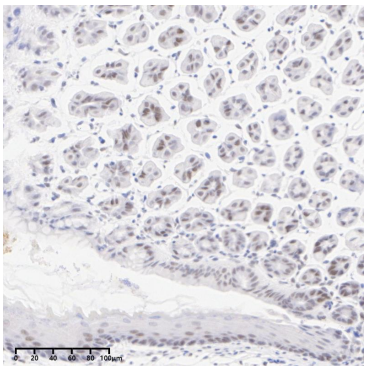

Fig8: Immunohistochemical analysis of paraffin-embedded mouse stomach tissue with Rabbit anti-Dnmt1 antibody (ET1702-77) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) 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 (ET1702-77) 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.

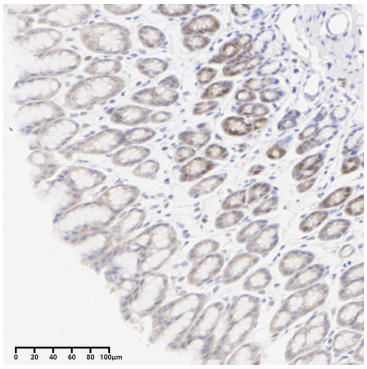

Fig9: Immunohistochemical analysis of paraffin-embedded rat stomach tissue with Rabbit anti-Dnmt1 antibody (ET1702-77) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) 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 (ET1702-77) 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.

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

Background References

1. Liu R et al. Dnmt1 regulates the myogenic lineage specification of muscle stem cells. Sci Rep 6:35355 (2016).
2. Chalertpet K et al. Human papillomavirus type 16 E7 oncoprotein mediates CCNA1 promoter methylation. Cancer Sci 106:1333-40 (2015).