Anti-PMS2 Antibody [SY08-09]

ET1605-1



Product Type: Recombinant Rabbit monoclonal IgG, primary antibodies

Species reactivity: Human

Applications: WB, IF-Cell, IF-Tissue, IHC-P, IP, FC

Molecular Wt: Predicted band size: 96 kDa

Clone number: SY08-09

Description: This gene is one of the PMS2 gene family members which are found in clusters on

chromosome 7. Human PMS2 related genes are located at bands 7p12, 7p13, 7q11, and 7q22. Exons 1 through 5 of these homologues share high degree of identity to human PMS2. The product of this gene is involved in DNA mismatch repair. The protein forms a heterodimer with MLH1 and this complex interacts with MSH2 bound to mismatched bases. Defects in this gene are associated with hereditary nonpolyposis colorectal cancer, with Turcot syndrome, and are a cause of supratentorial primitive neuroectodermal tumors.

Alternatively spliced transcript variants have been observed.

Immunogen: Synthetic peptide within Human PMS2 aa 1-50 / 862.

Positive control: HeLa, HeLa cell lysate, Jurkat cell lysate, HepG2 cell lysate, U-2 OS cell lysate, human

breast carcinoma tissue, human colon carcinoma tissue.

Subcellular location: Nucleus.

Database links: SwissProt: P54278 Human

Recommended Dilutions:

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

IP Use at an assay dependent concentration.

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°C long term.

Purity: Protein A affinity purified.

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Images

PMS2

OAPI

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Secondary antibody only control in HeLA cells

Secondary antibody crity control in HCT116 cells

Fig1: Immunocytochemistry analysis of HeLa (positive) and HCT 116 (negative) cells labeling PMS2 with Rabbit anti-PMS2 antibody (ET1605-1) at 1/200 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-PMS2 antibody (ET1605-1) at 1/200 dilution in 1% BSA in PBST overnight at 4 $^{\circ}$ C. 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 $+4^{\circ}$ C. Goat Anti-Mouse IgG H&L (iFluor \pm 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

Fig2: Western blot analysis of PMS2 on different lysates with Rabbit anti-PMS2 antibody (ET1605-1) at 1/10,000 dilution and competitor's antibody at 1/10,000 dilution.

Lane 1: HeLa cell lysate (20 µg/Lane) Lane 2: Jurkat cell lysate (20 µg/Lane)

Lane 3: HCT 116 cell lysate (negative) (20 µg/Lane)

Lane 4: HepG2 cell lysate (20 µg/Lane)

Predicted band size: 96 kDa Observed band size: 120 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 (ET1605-1) at 1/10,000 dilution and competitor's antibody at 1/10,000 dilution were used in 5% NFDM/TBST at $4\,^{\circ}$ C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

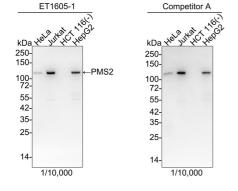


Fig3: Western blot analysis of PMS2 on different lysates with Rabbit anti-PMS2 antibody (ET1605-1) at 1/2,000 dilution.

Lane 1: HeLa cell lysate Lane 2: U-2 OS cell lysate Lane 3: Jurkat cell lysate Lane 4: HepG2 cell lysate

Lane 5: HCT 116 cell lysate (negative)

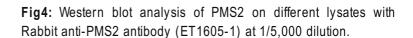
Lysates/proteins at 20 µg/Lane.

Predicted band size: 96 kDa Observed band size: 120 kDa

Exposure time: 37 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 (ET1605-1) at 1/2,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/100,000 dilution was used for 1 hour at room temperature.



Lane 1: HAP1-parental cell lysate Lane 2: HAP1-PMS2 KD cell lysate

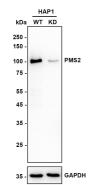
Lysates/proteins at 10 µg/Lane.

Predicted band size: 96 kDa Observed band size: 100 kDa

Exposure time: 1 minute; 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 (ET1605-1) at 1/5,000 dilution was used in primary antibody dilution (K1803) at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.



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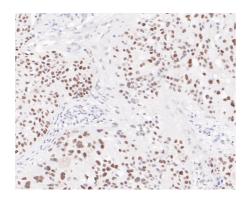


Fig5: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using anti-PMS2 antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH $_2$ O and PBS, and then probed with the primary antibody (ET1605-1, 1/200) for 30 minutes 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.

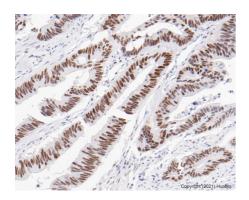


Fig6: Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue using anti-PMS2 antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET1605-1, 1/50) for 30 minutes 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.

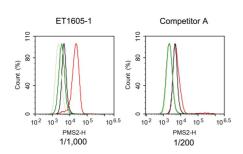


Fig7: Flow cytometric analysis of HeLa (positive, red) and HCT 116 (negative, green) cells labeling PMS2.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1605-1, red) at 1/1,000 dilution and competitor's antibody (red) at 1/200 dilution, compared with Rabbit IgG Isotype Control (HeLa black, HCT 116 light green). After incubation of the primary antibody at +4 $^{\circ}$ C for an hour, the cells were stained with a iFluor TM 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4 $^{\circ}$ C.

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

Background References

- 1. Wielders EA et al. Functional analysis of MSH2 unclassified variants found in suspected Lynch syndrome patients reveals pathogenicity due to attenuated mismatch repair. J Med Genet 51:245-53 (2014).
- 2. Joost P et al. Heterogenous mismatch-repair status in colorectal cancer. Diagn Pathol 9:126 (2014).

