Anti-MLH1 Antibody [A9F7]

HA601135

Product Type:	Mouse monoclonal IgG2b, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P
Molecular Wt:	Predicted band size: 85 kDa
Clone number:	A9F7

Description: DNA mismatch repair protein Mlh1 or MutL protein homolog 1 is a protein that in humans is encoded by the MLH1 gene located on chromosome 3. It is a gene commonly associated with hereditary nonpolyposis colorectal cancer. Orthologs of human MLH1 have also been studied in other organisms including mouse and the budding yeast Saccharomyces cerevisiae. This gene was identified as a locus frequently mutated in hereditary nonpolyposis colon cancer. It is a human homolog of the E. coli DNA mismatch repair gene, mutL, which mediates protein-protein interactions during mismatch recognition, strand discrimination, and strand removal. Defects in MLH1 are associated with the microsatellite instability observed in hereditary nonpolyposis colon cancer. Alternatively spliced transcript variants encoding different isoforms have been described, but their full-length natures have not been determined.

Immunogen: Recombinant protein within human MLH1 aa 452-751 / 751.

Positive control: HeLa cell lysate, A549 cell lysate, HepG2 cell lysate, Jurkat cell lysate, NIH/3T3 cell lysate, F9 cell lysate, RAW264.7 cell lysate, C6 cell lysate, A431 cell lysate, Daudi cell lysate, K-562 cell lysate, HL-60 cell lysate, SW480 cell lysate, human colon carcinoma tissue, mouse colon tissue, rat small intestine tissue.

Subcellular location: Nucleus, Chromosome.

Database links: SwissProt: P40692 Human | Q9JK91 Mouse | P97679 Rat

Recommended Dilutions:

WB	1:1,000-1:2,000
IHC-P	1:1,000

Storage Buffer: PBS (pH7.4), 0.1% 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

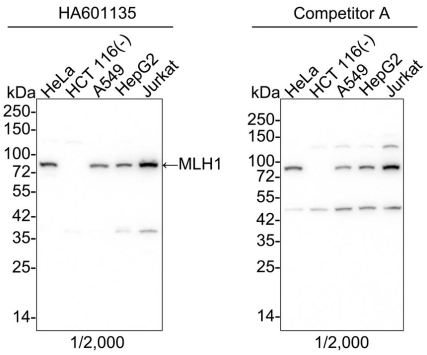

Fig1: Western blot analysis of MLH1 on different lysates with Mouse anti-MLH1 antibody (HA601135) at 1/2,000 dilution and competitor's antibody at 1/2,000 dilution.

Lane 1: HeLa cell lysate (15 µg/Lane)
Lane 2: HCT 116 cell lysate (negative) (15 µg/Lane)
Lane 3: A549 cell lysate (15 µg/Lane)
Lane 4: HepG2 cell lysate (15 µg/Lane)
Lane 5: Jurkat cell lysate (15 µg/Lane)

Predicted band size: 85 kDa
Observed band size: 85 kDa

Exposure time: Lane 1-5 (left): 24 seconds; Lane 1-5 (right): 2 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 (HA601135) at 1/2,000 dilution and competitor's antibody at 1/2,000 dilution were 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.

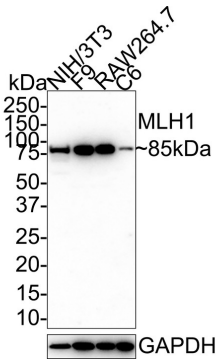

Fig2: Western blot analysis of MLH1 on different lysates with Mouse anti-MLH1 antibody (HA601135) at 1/1,000 dilution.

Lane 1: NIH/3T3 cell lysate (20 µg/Lane)
Lane 2: F9 cell lysate (20 µg/Lane)
Lane 3: RAW264.7 cell lysate (20 µg/Lane)
Lane 4: C6 cell lysate (20 µg/Lane)

Predicted band size: 85 kDa
Observed band size: 85 kDa

Exposure time: 3 minutes 10 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 (HA601135) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1:50,000 dilution was used for 1 hour at room temperature.

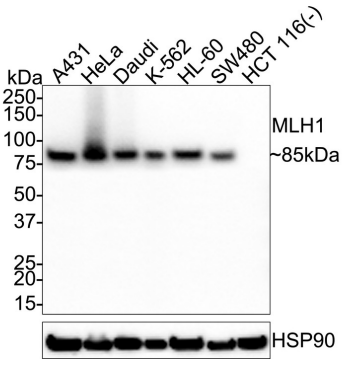

Fig3: Western blot analysis of MLH1 on different lysates with Mouse anti-MLH1 antibody (HA601135) at 1/1,000 dilution.

Lane 1: A431 cell lysate (20 µg/Lane)
Lane 2: HeLa cell lysate (20 µg/Lane)
Lane 3: Daudi cell lysate (20 µg/Lane)
Lane 4: K-562 cell lysate (20 µg/Lane)
Lane 5: HL-60 cell lysate (20 µg/Lane)
Lane 6: SW480 cell lysate (20 µg/Lane)
Lane 7: HCT 116 cell lysate (Negative) (20 µg/Lane)

Predicted band size: 85 kDa
Observed band size: 85 kDa

Exposure time: 20 seconds;

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 (HA601135) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1:150,000 dilution was used for 1 hour at room temperature.

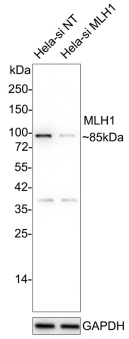

Fig4: Western blot analysis of MLH1 on different lysates with Mouse anti-MLH1 antibody (HA601135) at 1/2,000 dilution.

Lane 1: Hela-si NT cell lysate
Lane 2: Hela-si MLH1 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 85 kDa
Observed band size: 85 kDa

Exposure time: 50 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

HA601135 was shown to specifically react with MLH1 in Hela-si NT cells. Weakened band was observed when Hela-si MLH1 sample was tested. Hela-si NT and Hela-si MLH1 samples were subjected to SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary antibody (HA601135, 1/2,000) and Loading control antibody (Rabbit anti-GAPDH, ET1601-4, 1/10,000) were 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.

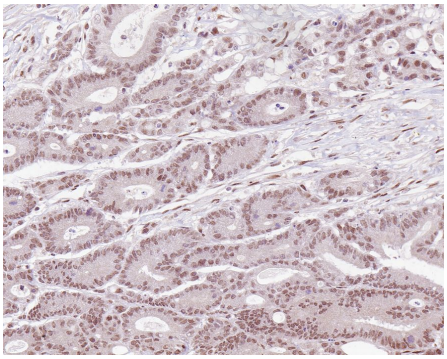

Fig5: Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue with Mouse anti-MLH1 antibody (HA601135) at 1/1,000 dilution.

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 (HA601135) 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.

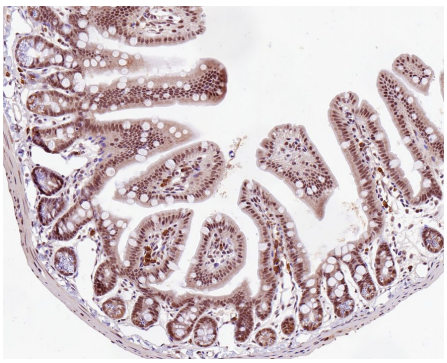

Fig6: Immunohistochemical analysis of paraffin-embedded mouse colon tissue with Mouse anti-MLH1 antibody (HA601135) at 1/1,000 dilution.

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 (HA601135) 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.

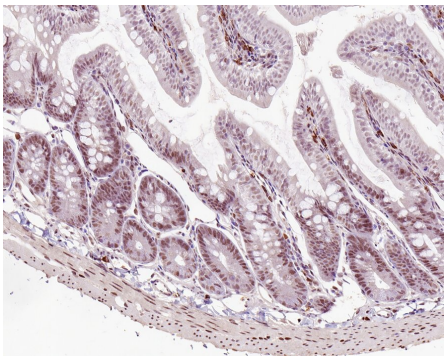

Fig7: Immunohistochemical analysis of paraffin-embedded rat small intestine tissue with Mouse anti-MLH1 antibody (HA601135) at 1/1,000 dilution.

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 (HA601135) 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.

Background References

1. Guan J et al. MLH1 Deficiency-Triggered DNA Hyperexcision by Exonuclease 1 Activates the cGAS-STING Pathway. Cancer Cell. 2021 Jan
2. Cannavo E et al. Regulation of the MLH1-MLH3 endonuclease in meiosis. Nature. 2020 Oct