Anti-MLH1 Antibody [SP08-04] - BSA and Azide free

HA750077

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: 85 kDa
Clone number:	SP08-04

Description: DNA-mismatch repair (MMR) is an essential process in maintaining genetic stability. Lack of a functional DNA-mismatch repair pathway is a common characteristic of several different types of human cancers, either due to an MMR gene mutation or promoter methylation gene silencing. MLH1 is an integral part of the protein complex responsible for mismatch repair and is expressed in lymphocytes, heart, colon, breast, lung, spleen, testis, prostate, thyroid and gall bladder tissues, and is methylated in several ovarian tumors. Loss of MLH1 protein expression is associated with a mutated phenotype, microsatellite instability and a predisposition to cancer. In hereditary nonpolyposis colorectal cancer (HNPCC), an autosomal dominant inherited cancer syndrome that signifies a high risk of colorectal and various other types of cancer, the MLH1 gene exhibits a pathogenic mutation. Certain cancer cell lines, including leukemia CCRF-CEM, colon HCT 116 and KM12, and ovarian cancers SK-OV-3 and IGROV-1, show complete deficiency of MLH1, while MLH1 is expressed in 60% of melanomas, 70% of noninvasive squamous cell carcinomas and 30% of invasive squamous cell carcinomas.

Immunogen: Synthetic peptide within Human MLH1 aa 707-756 / 756.

Positive control: B16F10 cell lysate, HeLa cell lysate, A549 cell lysate, MCF7 cell lysate, HepG2 cell lysate, HepG2, rat large intestine tissue, human tonsil tissue, mouse testis tissue, Hela.

Subcellular location: Nucleus.

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

Recommended Dilutions:

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

Storage Buffer: PBS (pH7.4).

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

Purity: Protein A affinity purified.

Images

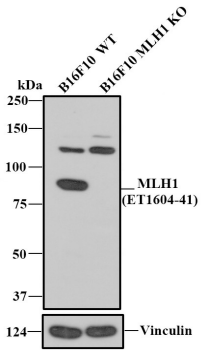

Fig1: All lanes: Western blot analysis of MLH1 with anti-MLH1 antibody [SP08-04] (HA750077) at 1:1,000 dilution.

Lane 1: Wild-type B16F10 whole cell lysate.
Lane 2: MLH1 knockout B16F10 whole cell lysate.

ET1604-41 was shown to specifically react with MLH1 in wild-type B16F10 cells. No band was observed when MLH1 knockout samples were tested. Wild-type and MLH1 knockout 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 Anti-MLH1 antibody (ET1604-41, 1/1,000) and Anti-Vinculin antibody (ET1705-94, 1/5,000) were used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG H&L (HRP) Secondary Antibody (HA1001) at 1:200,000 dilution was used for 1 hour at room temperature.

Cell lysate was provided by Ubigene Biosciences (Ubigene Biosciences Co., Ltd., Guangzhou, China).

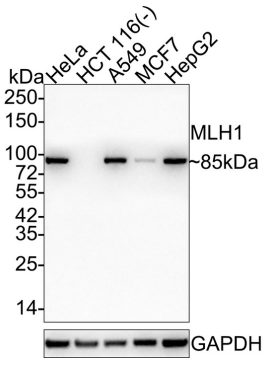

Fig2: Western blot analysis of MLH1 on different lysates with Rabbit anti-MLH1 antibody (HA750077) at 1/5,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HCT 116 cell lysate (negative)
Lane 3: A549 cell lysate
Lane 4: MCF7 cell lysate
Lane 5: HepG2 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 85 kDa
Observed band size: 85 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 (HA750077) at 1/5,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

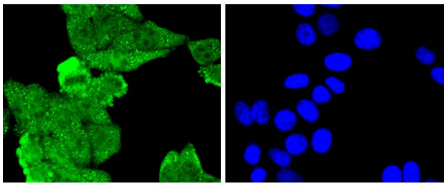

Fig3: ICC staining of MLH1 in HepG2 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (HA750077, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

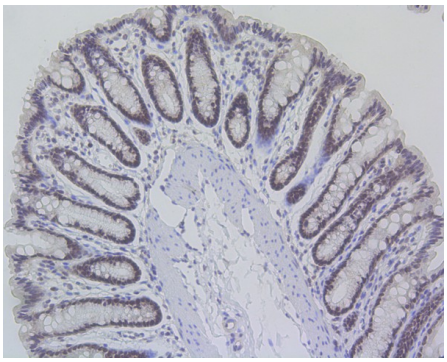

Fig4: Immunohistochemical analysis of paraffin-embedded rat large intestine tissue using anti-MLH1 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750077, 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.

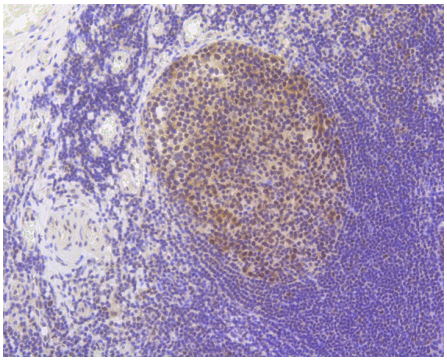

Fig5: Immunohistochemical analysis of paraffin-embedded human tonsil tissue using anti-MLH1 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750077, 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.

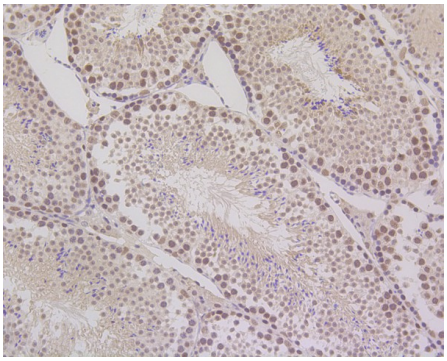

Fig6: Immunohistochemical analysis of paraffin-embedded mouse testis tissue using anti-MLH1 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750077, 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.

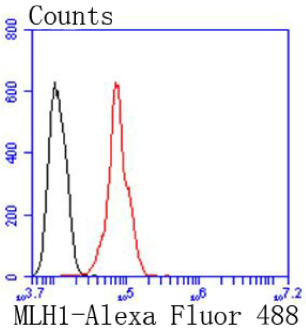

Fig7: Flow cytometric analysis of MLH1 was done on Hela cells. The cells were fixed, permeabilized and stained with the primary antibody (HA750077, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Rabbit IgG Secondary antibody at 1/1000 dilution for 30 minutes.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. Yue Q et al. Histone H3K9 Lactylation Confers Temozolomide Resistance in Glioblastoma via LUC7L2-Mediated MLH1 Intron Retention. Adv Sci (Weinh). 2024 May
2. Guan J et al. MLH1 Deficiency-Triggered DNA Hyperexcision by Exonuclease 1 Activates the cGAS-STING Pathway. Cancer Cell. 2021 Jan