Anti-MSH2 Antibody [10G1]

EM1801-04

Product Type:	Mouse monoclonal IgG2b, primary antibodies
Species reactivity:	Human, Rat, Mouse
Applications:	WB, IHC-P
Molecular Wt:	Predicted band size: 105 kDa
Clone number:	10G1

Description: Component of the post-replicative DNA mismatch repair system (MMR). Forms two different heterodimers: MutS alpha (MSH2-MSH6 heterodimer) and MutS beta (MSH2-MSH3 heterodimer) which binds to DNA mismatches thereby initiating DNA repair. When bound, heterodimers bend the DNA helix and shields approximately 20 base pairs. MutS alpha recognizes single base mismatches and dinucleotide insertion-deletion loops (IDL) in the DNA. MutS beta recognizes larger insertion-deletion loops up to 13 nucleotides long. After mismatch binding, MutS alpha or beta forms a ternary complex with the MutL alpha heterodimer, which is thought to be responsible for directing the downstream MMR events, including strand discrimination, excision, and resynthesis. Recruits DNA helicase MCM9 to chromatin which unwinds the mismatch containg DNA strand. ATP binding and hydrolysis play a pivotal role in mismatch repair functions. The ATPase activity associated with MutS alpha regulates binding similar to a molecular switch: mismatched DNA provokes ADP-->ATP exchange, resulting in a discernible conformational transition that converts MutS alpha into a sliding clamp capable of hydrolysis-independent diffusion along the DNA backbone. This transition is crucial for mismatch repair. MutS alpha may also play a role in DNA homologous recombination repair. In melanocytes may modulate both UV-B-induced cell cycle regulation and apoptosis.

Immunogen:	Synthetic peptide within N-terminal human MSH2.
Positive control:	HeLa cell lysate, HEK-293 cell lysate, A549 cell lysate, mouse testis tissue lysate, rat testis tissue lysate, Wild-type SCC7 whole cell lysates, human breast carcinoma tissue, human placenta tissue, rat brain tissue, human kidney tissue.
Subcellular location:	Nucleus, Chromosome.
Database links:	SwissProt: P43246 Human | P43247 Mouse | P54275 Rat
Recommended Dilutions:	
WB	1:2,000-1:5,000
IHC-P	1:100-1:1,000
Storage Buffer:	1*PBS (pH7.4), 0.2% BSA, 50% Glycerol. Preservative: 0.05% Sodium Azide.
Storage Instruction:	Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.
Purity:	Protein G affinity purified.

Images

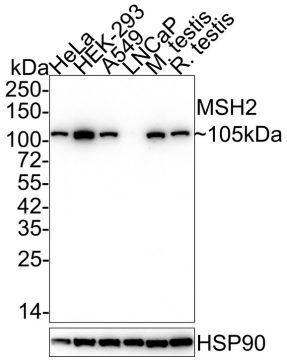

Fig1: Western blot analysis of MSH2 on different lysates with Mouse anti-MSH2 antibody (EM1801-04) at 1/5,000 dilution.

Lane 1: HeLa cell lysate (15 µg/Lane)
Lane 2: HEK-293 cell lysate (15 µg/Lane)
Lane 3: A549 cell lysate (15 µg/Lane)
Lane 4: LNCAP cell lysate (negative) (15 µg/Lane)
Lane 5: Mouse testis tissue lysate (20 µg/Lane)
Lane 6: Rat testis tissue lysate (20 µg/Lane)

Predicted band size: 105 kDa
Observed band size: 105 kDa

Exposure time: 2 minutes 18 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 (EM1801-04) at 1/5,000 dilution was 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.

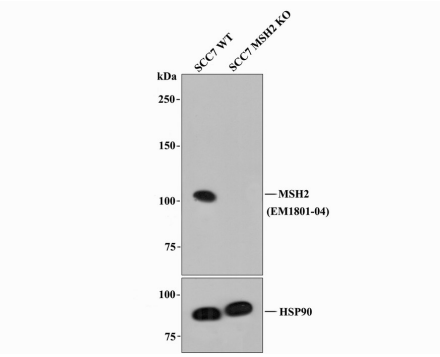

Fig2: All lanes: Western blot analysis of MSH2 with anti-MSH2 antibody (EM1801-04) at 1/1,000 dilution.
Lane 1: Wild-type SCC7 whole cell lysate.
Lane 2: MSH2 knockout SCC7 whole cell lysate.

EM1801-04 was shown to specifically react with MSH2 in Wild-type SCC7 cells. No band was observed when MSH2 knockout sample was tested. Wild-type and MSH2 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-MSH2 antibody (EM1801-04, 1/1,000) and Anti-HSP90 antibody (ET1605-56, 1/10,000) were used in 5% BSA at room temperature for 2 hours. Goat Anti-Mouse IgG HRP Secondary Antibody (HA1006) at 1:20,000 dilution was used for 1 hour at room temperature.

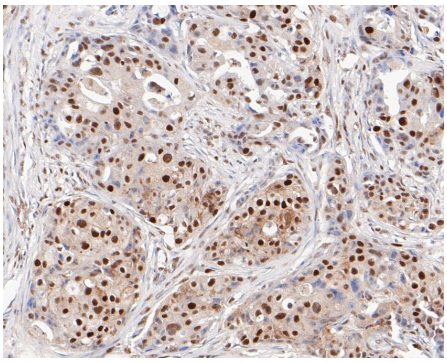

Fig3: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using anti-MSH2 antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (EM1801-04, 1/800) 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.

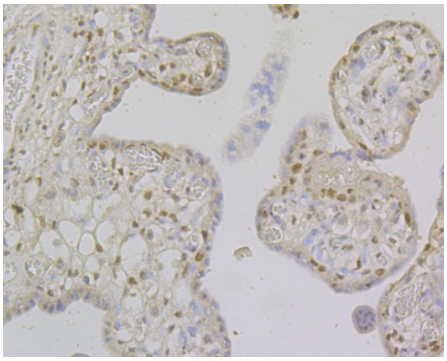

Fig4: Immunohistochemical analysis of paraffin-embedded human placenta tissue using anti-MSH2 antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (EM1801-04, 1/400) 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.

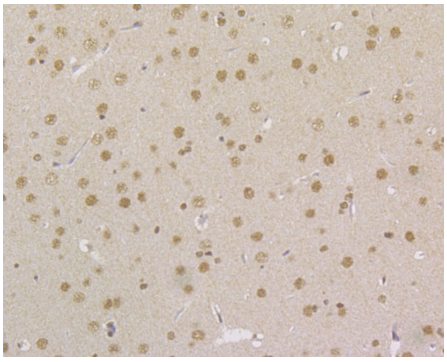

Fig5: Immunohistochemical analysis of paraffin-embedded rat brain tissue using anti-MSH2 antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (EM1801-04, 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.

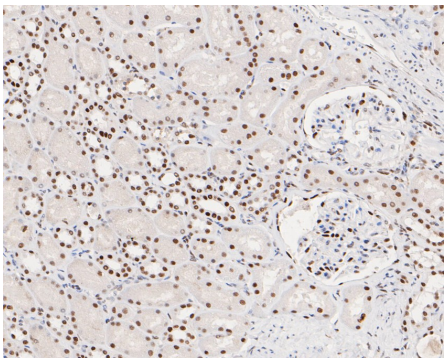

Fig6: Immunohistochemical analysis of paraffin-embedded human kidney tissue using anti-MSH2 antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (EM1801-04, 1/800) 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.

Background References

1. Kansikas M. et al. Verification of the three-step model in assessing the pathogenicity of mismatch repair gene variants. Hum. Mutat. 32:107-115(2011).
2. Traver S. et al. MCM9 Is Required for Mammalian DNA Mismatch Repair. Mol. Cell 59:831-839(2015).