

Anti-Phospho-MCM2 (S40) Antibody [JE35-01]

HA722216



Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, FC, IF-Tissue
Molecular Wt:	Predicted band size: 102 kDa
Clone number:	JE35-01

Description: DNA replication licensing factor MCM2 is a protein that in humans is encoded by the MCM2 gene. The protein encoded by this gene is one of the highly conserved mini-chromosome maintenance proteins (MCM) that are involved in the initiation of eukaryotic genome replication. The hexameric protein complex formed by MCM proteins is a key component of the pre-replication complex (pre-RC) and may be involved in the formation of replication forks and in the recruitment of other DNA replication-related proteins. This protein forms a complex with MCM4, 6, and 7, and has been shown to regulate the helicase activity of the complex. This protein is phosphorylated, and thus regulated by, protein kinases CDC2 and CDC7.

Immunogen: Synthetic phosphopeptide corresponding to residues surrounding Ser40 of human MCM2.

Positive control: HeLa cell lysate, 293T cell lysate, NIH/3T3 cell lysate, C6 cell lysate, HeLa, human tonsil tissue, mouse spleen tissue, rat spleen tissue.

Subcellular location: Nucleus, Chromosome.

Database links: SwissProt: P49736 Human | P97310 Mouse
Entrez Gene: 312538 Rat

Recommended Dilutions:

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

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

Storage Instruction: Shipped at 4°C. Store at +4°C 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.

Hangzhou Huaan Biotechnology Co., Ltd.

Orders:0086-571-88062880

Technical:0086-571-89986345

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Images

Fig1: Western blot analysis of Phospho-MCM2 (S40) on different lysates with Rabbit anti-Phospho-MCM2 (S40) antibody (HA722216) at 1/2,000 dilution.

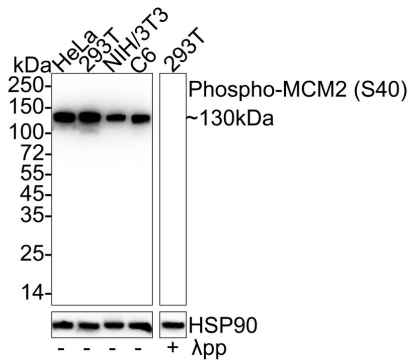
Lane 1: HeLa cell lysate

Lane 2: 293T cell lysate

Lane 3: NIH/3T3 cell lysate

Lane 4: C6 cell lysate

Lane 5: 293T cell lysate, the membrane treated with λ pp for 1 hour



Lysates/proteins at 20 μ g/Lane.

Predicted band size: 102 kDa

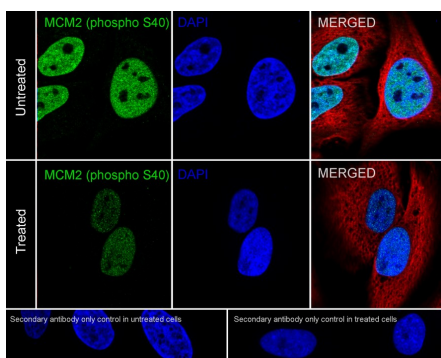
Observed band size: 130 kDa

Exposure time: 14 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 (HA722216) at 1/2,000 dilution was used in 5% NFDM/TBST 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.

Fig2: Immunocytochemistry analysis of HeLa cells treated with or without λ pp labeling Phospho-MCM2 (S40) with Rabbit anti-Phospho-MCM2 (S40) antibody (HA722216) at 1/1,000 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-Phospho-MCM2 (S40) antibody (HA722216) at 1/1,000 dilution in 1% BSA in PBST overnight at 4°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°C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

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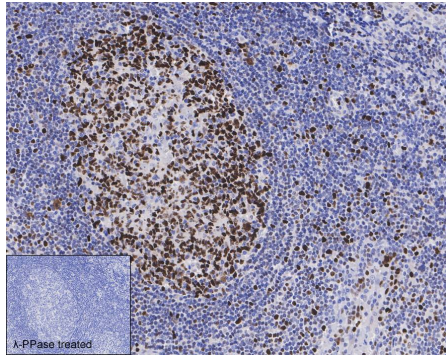


Fig3: Immunohistochemical analysis of paraffin-embedded human tonsil tissue untreated / treated with λ pp with Rabbit anti-Phospho-MCM2 (S40) antibody (HA722216) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (HA722216) 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.

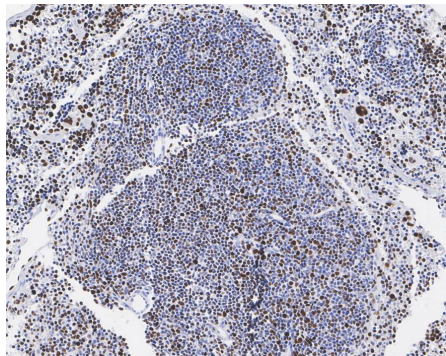


Fig4: Immunohistochemical analysis of paraffin-embedded mouse spleen tissue with Rabbit anti-Phospho-MCM2 (S40) antibody (HA722216) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (HA722216) 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.

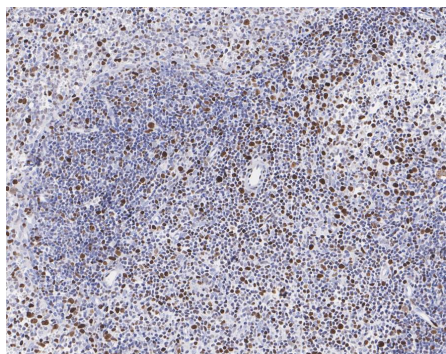


Fig5: Immunohistochemical analysis of paraffin-embedded rat spleen tissue with Rabbit anti-Phospho-MCM2 (S40) antibody (HA722216) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (HA722216) 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.

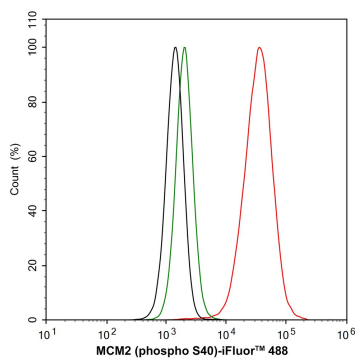


Fig6: Flow cytometric analysis of HeLa cells labeling Phospho-MCM2 (S40).

Cells were fixed and permeabilized. Then stained with the primary antibody (HA722216, 1 μ g/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4 $^{\circ}$ C for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4 $^{\circ}$ C. 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. Sun Y et al. MCM2 in human cancer: functions, mechanisms, and clinical significance. Mol Med. 2022 Oct
2. Zhou X et al. MCM2 promotes the stemness and sorafenib resistance of hepatocellular carcinoma cells via hippo signaling. Cell Death Discov. 2022 Oct

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