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AM26592AF-N Monoclonal Antibody to MCM3 (full length) - Azide Free

Alternate names: DNA polymerase alpha holoenzyme-associated protein P1, DNA replication licensing factor MCM3, P1-MCM3, P102 protein, RLF subunit beta, mini-chromosome maintenance protein 3

Quantity: 0.1 mg

Concentration: 1.0 mg/ml (after reconstitution)

Background: MCM3 is a member of the MCM protein family which are essential for the initiation of DNA replication. Although definite functions of the MCM proteins remain largely unknown, they have been implicated in the regulatory machinery allowing DNA to replicate only once in the S phase. The MCM proteins are present at relatively constant throughout the cell cycle in actively proliferating cells, but bind chromatin in a cell cycle specific manner. MCM pr oteins bind to chromatin, before the cell cycle enters the S phase, and make chromatin competent for replication. During replication, MCM proteins dissociate from chromatin in a process that is accompanied by phosphorylation of several members of the MCM family. Replicated chromatin lacks bound MCM proteins and it is therefore unable to replicate within the same cell cycle. MCM3 is expressed throughout the cell cycle (G 1 , S, G 2 , M phases), but not in resting (G 0) cells. Because of its expression, MCM3 can be used for cell proliferation marker.

Uniprot ID: P25205

NCBI: NP_002379.2

GeneID: 4172

Host / Isotype: Mouse / IgG2a

Clone: 3A2

Immunogen: Recombinant full-length human MCM3

Format:
State: Lyophilized Ig fraction
Purification: Protein A agarose.
Buffer System:

PBS (pH 7.2) / 1% sucrose. No preservative is contained.

Reconstitution: Restore with 100 µl distilled water.

Applications:
Western blot: 1 µg/ml for chemiluminescence detection system.
Immunoprecipitation: 1-5 µg/200-300 µl of cell extract.
Immunohistochemistry on paraffin sections: 5 µg/ml (heat treatment is necessaray; microwave oven; 2 times for 10 minutes each in 10 mM citrate buffer (pH6.5).
Immunocytochemistry: 10 µg/ml.
For details se protocols below.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: This antibody reacts with MCM3.

Species Reactivity: **Tested:** Human (Jurkat, Raji, HeLa, MRC-5, A431, etc.). Does not react with Mouse (NIH/3T3, WR19L).

Add. Information: This product was originally produced by MBL International.

Storage: Prior to reconstitution store at 2-8°C.
Following reconstitution store undiluted at -20°C.
Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

General Readings:

1. Srsen, V., et al ., J . Cell Biol . 174 , 625-630 (2006).
2. Sato, N., et al ., Genes Cells 8 , 451-463 (2003).
3. Tsuruga, H., et al ., Genes Cells 2 , 381-399 (1997).
4. Dutta, A., et al., Annu. Rev. Cell Dev Biol . 13 , 293 –332 (1997).
5. Young, M., et al., Mol. Cell. Biol . 8 , 1587-1601 (1997).
6. Tsuruga, H., et al ., Biochem . Biophys . Res . Commun . 236 , 118-125 (1997).
7. Kubota, Y., et al., EMBO J . 16 , 3320-3331 (1997).
8. Kubota, Y., et al., Cell 81 , 601-609 (1995).
9. Chong, J., et al., Nature 375 , 418-421 (1995).

Protocols:

SDS-PAGE & Western Blotting
1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4 o C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4 o C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the Lysis buffer to make 8 mg/mL solution.
3) Mix the sample with equal volume of Laemmli's sample buffer.
4) Boil the samples for 2 minutes and centrifuge. Load 10 µ L of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm 2 for 1 hour in a semi-dry transfer system. (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
6) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggest in the APPLICATIONS for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
9) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS-T (5 minutes x 6 times).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemilumin escence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
12) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; human cell lines)

Immunoprecipitation
1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4 o C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4 o C and transfer the supernatant to another tube.
3) Add primary antibody as suggest in the APPLICATIONS into 250 µ L of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4 o C. Add 20 µ L of 50% protein A agarose beads resuspended in the Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4 o C.
4) Wash the beads 3-5 times with ice-cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
5) Resuspend the beads in 20 µ L of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10 µ L/lane for the SDS-PAGE analysis. (See SDS-PAGE & Western blotting.)
(Positive controls for Immunoprecipitation; human cell lines)

Immunohistochemical staining for paraffin-embedded sections : SAB method
1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
2) Wash the slides with Ethanol 3 times for 3-5 minutes each.
3) Wash the slides with PBS 3 times for 3-5 minutes each.
4) Heat treatment Heat treatment by microwave oven: Place the slides put on staining basket in 500 mL beaker with 500 mL of 10 mM citrate buffer (pH 6.5). Cover the beaker with plas tic wrap, then process the slides 2 times for 10 minutes each at 500 W with microwave oven. Let the slides cool down in the beaker at room temperature for about 40 minutes.
5) Remove the slides from the citrate buffer and cover each section with 3% H 2 O 2 for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 3 times in PBS for 5 minutes each.
6) Remove the slides from PBS, wipe gently around each section and cover tissues with Protein Blocking Agent for 5 minutes to block non-specific staining. Do not wash.
7) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with PBS containing 1% BSA as suggest in the APPLICATIONS.
8) Incubate the sections for 1 hour at room temperature.
9) Wash the slides 3 times in PBS for 5 minutes each.
10) Wipe gently around each section and cover tissues with Polyvalent Biotinylated Antibody. Incubate for 10 minutes at room temperature. Wash as in step 9).
11) Wipe gently around each section and cover tissues with Streptavidin-Peroxidase. Incubate for 10 minutes at room temper ature. Wash as in step 9).
12) Visualize by reacting for 10-20 minutes with substrate solution containing 7.5 mg DAB, 40 µ L of 30% H 2 O 2 in 150 mL PBS. * DAB is a suspect carcinogen and must be handled with care. Always wear gloves.

13) Wash the slides in water for 5 minutes.
14) Counter stain in hematoxylin for 1 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes. Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each. 15) Now ready for mounting.
(Positive controls for Immunohistochemistry; human cell lines)

Immunocytochemistry
1) Culture the cells in the appropriate condition on a glass slide. (for example, spread 10 4 of HeLa cells for one slide, then incubate in a CO 2 incubator for one night.)
2) Rinse the cells on glass coverslips in PBS and fix cells by immersing acetone, air dry.
3) Cover the cells with blocking buffer (0.2 % BSA or 10 % normal goat serum in PBS) for 10 minutes to minimize non-specific adsorption of the antibodies to the cover slip (25-50 µ L is usually sufficient).
4) Remove the blocking buffer.
5) Add the primary antibody diluted with blocking buffer as suggest in the APPLICATIONS onto the cells and incubate for 1 hour at room temperature.
(Optimization of antibody concentration or incubation condition are recommended if necessary.)
6) Prepare a wash container such as a 500 mL beaker with a stirrer. Then wash the cultured cells on the glass slide by soaking the slide with a plenty of PBS in the wash container for 5 minutes. Take care not to touch the cells. Repeat another washes once more.
7) Add 100 µL of 1:40 diluted FITC conjugated goat anti-mouse IgG onto the cells. Incubate for 1 hour at room temperature. Keep out light by aluminum foil.
8) Wash the slide in a plenty of PBS as in the step 5).
9) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
10) Promptly add Permafluor TM aqueous mounting medium onto the slide, then put a cover slip on it.
(Positive controls for Imm unocytochemistry; human cell lines)

Pictures:

Western blot analysis of MCM3 expression in Jurkat (1), Raji (2), HeLa (3), MRC-5 (4), ZR-75-1 (5), Hep-II (6), HepG2 (7), A431 (8), NIH/3T3 (9) and WR19L (10) using AM26592AF-N.