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Product datasheet for AM26600AF-N

MCM7 (full length) Mouse Monoclonal Antibody [Clone ID: 4B4]

Product data:

Product Type:	Primary Antibodies
Clone Name:	4B4
Applications:	IF, WB
Recommended Dilution:	**Western blot:** 1 µg/ml for chemiluminescence detection system. **Immunocytochemistry:** 5 µg/ml.
Reactivity:	Human, Mouse
Host:	Mouse
Isotype:	IgG2a
Clonality:	Monoclonal
Immunogen:	Recombinant full-length human MCM7
Formulation:	PBS (pH 7.2)/1% sucrose. No preservative is added. State: Azide Free State: Lyophilized Ig fration
Reconstitution Method:	Restore with 100 µl distilled water.
Concentration:	lot specific
Purification:	Protein A agarose
Conjugation:	Unconjugated
Storage:	Prior to reconstitution store at 2-8°C. Following reconstitution store undiluted at -20°C. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Gene Name:	minichromosome maintenance complex component 7
Database Link:	Entrez Gene 4176 Human P33993

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Background: The replication of the large genome of eukaryotic cells is made possible by the use of multiple replication origins per chromosome. However, multiple origins must be strictly regulated if all chromosomal sequences are to be replicated only once in S phase, and re-replication of DNA before completing mitosis is prohibited. This characteristic is thought to be due to the properties of "initiation" proteins, such as Orc (Origin recognition complex), Cdc6 and MCM (Minichromosome maintenance) protein family. Experiments used with yeast and Xenopus had clearly s hown that the replication preinitiation complex is assembled in an ordered sequence as Orc recruits Cdc6, which in turn recruits MCM complex (consisting of MCM 2-7 proteins). MCM proteins occur in high copy numbers in nuclei of human cells. They appear to be either free in the nucleoplasm or bound to chromatin. The fraction of structure-bound mammalian MCM proteins is highest at the beginning of S phase, but gradually decreases during progression of replication.

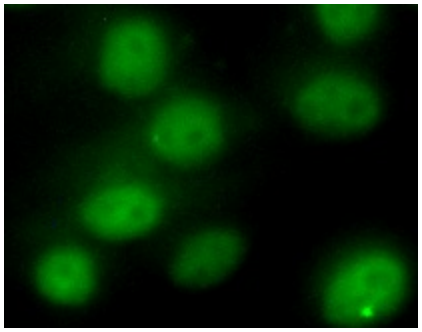
Synonyms: CDC47, MCM2, CDC47 homolog, P1.1-MCM3

Note: This product was originally produced by MBL International.

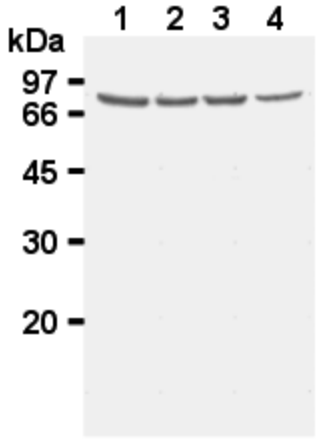
Protocol:
SDS-PAGE & Western Blotting
1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4 o C with rotating for 30 minutes, then sonicate 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; Jurkat, HeLa, Raji, WR19L)
Immunocytochemistry
1) Culture the cells in the appropriate condition on a glass slide. (for exampl e, spread 1x10 4 cells of HeLa cells for one slide, then incubate in a CO 2 incubator for one night.)
2) Rinse the cultured cells on the glass slide with Wash buffer (PBS containing 2% FCS).
3) Fix the cells by immersing the slide in PBS containing 4% paraformaldehyde (PFA)/PBS for 10 minutes at room temperature. Don't fix the cells with acetone. 4) The glass slide was washed with Wash buffer 3 times.
5) Immerse the slide in PBS containing 0.1% Triton X-100 for 20 minutes at 4 o C.
6) The glass slide was washed with Wash buffer 3 times.
7) Add the primary antibody dilute d with PBS as suggest in the APPLICATIONS onto the cells and incubate for 30 minutes at 4 o C. (Optimization of antibody concentration or incubation condition are recommended if necessary.)
8) The glass slide was washed with Wash buffer 3 times.
9) Add 100 µ L of 1:100 FITC conjugated anti-mouse IgG diluted with Wash buffer onto the cells. Incubate for 30 minutes at 4 o C. Keep out light by aluminum foil.
10) The glass slide was washed with Wash buffer 3 times.
11) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
12) Promptly add mounting medium onto the slide, then put a cover slip on it.
(Positive control for Immunocytochemistry; HeLa)

Product images:



Immunocytochemical detection of MCM7 on 4% PFA fixed HeLa cells with AM26600AF-N.



Western blot analysis of MCM7 expression in Jurkat (1), HeLa (2), Raji (3) and WR19L (4) using AM26600AF-N.