**For Research Use Only.**
**Not for use in diagnostic procedures.**

MONOCLONAL ANTIBODY

# Anti-MCM 7

| Code No. | Clone | Subclass | Quantity | Form |
|---|---|---|---|---|
| M049-3 | 4B4 | Mouse IgG2a | 100 µg | lyophilized |

**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 shown 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.

**SOURCE:** This antibody was purified from mouse ascites fluid using protein A agarose. This hybridoma (clone 4B4) was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse splenocyte immunized with the recombinant full-length human MCM7.

**FORMULATION:** This antibody is lyophilized form. Prepare a stock solution by dissolving the lyophilized antibody in 100 µL of distilled water. After reconstitution, the IgG concentration should be 1 mg/mL in PBS (pH 7.2)/1% sucrose. No preservative is contained.

**STORAGE:** This antibody is stable for one year from the date of shipment when stored at 4°C. After reconstitution, avoid repeated freezing and thawing. For storage, prepare appropriate aliquots and freeze them at -20°C.

**REACTIVITY:** This antibody reacts with human and mouse MCM7 on Western blotting and Immunocytochemistry.

**APPLICATIONS:**

Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; Not recommended
Immunohistochemistry; Not tested
Immunocytochemistry; 5 µg/mL
Flow cytometry; Not tested

Detailed procedure is provided in the following **PROTOCOLS**.

**SPECIES CROSS REACTIVITY:**

| Species | Human | Mouse | Rat |
|---|---|---|---|
| Cells | Jurkat, HeLa, Raji | WR19L | Not Tested |
| Reactivity on WB | + | + | |

**INTENDED USE:**

For Research Use Only. Not for use in diagnostic procedures.

**REFERENCES:**

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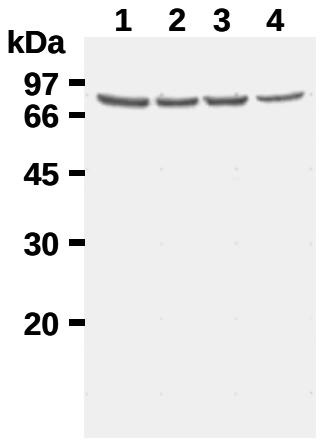
2) Fujii-Yamamoto, H., *et al., J. Biol. Chem.* **280**, 12976-12987 (2005)
3) Ohsaki, E., *et al., J. Virol*. **78**, 9936-9946 (2004)
4) Tatsumi, Y., *et al., J. Biol. Chem.* **278**, 41528-41534 (2003)
5) Sato, N., *et al., Genes Cells* **8**, 451-463 (2003)
6) Weinreich, M., *et al., PNAS* **96**, 441-446 (1999)
7) Ritzi, M., *et al., J. Biol. Chem*. **273**, 24543-24549 (1998)
8) Tanaka, T., *et al., EMBO J*. **17**, 5182-5191 (1998)
9) Kubota, Y., *et al., Cell* **81**, 601-609 (1995)
10) Chong, J., *et al., Nature* **375**, 418-421 (1995)
11) Madline, M. A., *et al., Nature* **375**, 421-424 (1995)

Clone 4B4 is used in reference number 1) - 5).




***Western blot analysis of MCM7 expression in Jurkat (1), HeLa (2), Raji (3) and WR19L (4) using M049-3.***

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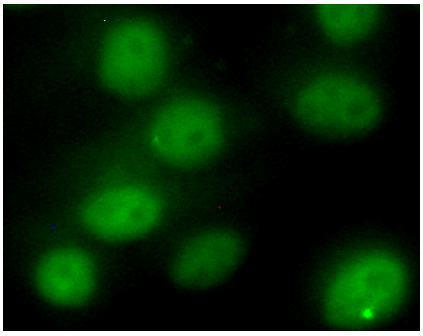
## PROTOCOLS:

### 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°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°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 μL 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 (MBL; code no. 330) 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 chemiluminescence 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)



***Immunocytochemical detection of MCM7 on 4% PFA fixed HeLa cells with M049-3.***

### Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (for example, 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°C.
6) The glass slide was washed with Wash buffer 3 times.
7) Add the primary antibody diluted with PBS as suggest in the **APPLICATIONS** onto the cells and incubate for 30 minutes at 4°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 μL of 1:100 FITC conjugated anti-mouse IgG (MBL; code no. IM-0819) diluted with Wash buffer onto the cells. Incubate for 30 minutes at 4°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)

## RELATED PRODUCTS:

| | |
|---|---|
| K0162-3 | anti-Cyclin A (E23.1) |
| K0163-3 | anti-Cyclin A (E67.1) |
| K0163-6 | Biotin labeled anti-Cyclin A (E67.1) |
| K0128-3 | anti-Cyclin B1 (V152) |
| K0164-3 | anti-Cyclin B1 (V92.1) |
| K0189-3 | anti-Cyclin B2 (X121.10) |
| 553 | anti-Cyclin D1 (polyclonal) |
| MD-17-3 | anti-Cyclin D1 (5D4) |
| MD-17-3H | anti-Cyclin D1 (5D4) |
| K0062-3 | anti-Cyclin D1 (DCS-6) |
| K0063-3 | anti-Cyclin D2 (DCS-3) |

| | |
|---|---|
| K0064-3 | anti-Cyclin D2 (DCS-5) |
| K0013-3 | anti-Cyclin D3 (DCS-22) |
| K0172-3 | anti-Cyclin E (HE12) |
| K0173-3 | anti-Cyclin E (HE172) |
| MT-19-3 | anti-Cdc2Hs (5F6) |
| K0069-3 | anti-CDC6 (DCS-180) |
| K0070-3 | anti-CDC7 (DCS-342) |
| CY-M1021 | anti-Phospho-Cdc7 Thr376 (TK-3H7) |
| K0140-3 | anti-Cdc20 (AR12) |
| K0071-3 | anti-CDC25A (DCS-120) |
| K0072-3 | anti-CDC25A (DCS-121) |
| K0073-3 | anti-CDC25A (DCS-124) |
| CY-1352 | CycLex® Cdc25A Protein Phosphatase Fluorometric Assay Kit |
| CY-E1352 | Recombinant Cdc25A (Catalytic Domain) |
| CY-1353 | CycLex® Cdc25B Protein Phosphatase Fluorometric Assay Kit |
| CY-E1353 | Recombinant Cdc25B (Catalytic Domain) |
| K0075-3 | anti-CDC25C (DCS-193) |
| K0200-3 | anti-Cdc25C (TC14) |
| CY-M1018 | anti-Phospho-Cdc25C Ser216 (TK-1F1) |
| CY-1354 | CycLex® Cdc25C Protein Phosphatase Fluorometric Assay Kit |
| CY-E1354 | Recombinant Cdc25C (Catalytic Domain) |
| CY-1355 | CycLex® Cdc25 Combo Protein Phosphatase Fluorometric Assay Kit |
| K0141-3 | anti-CDC27 (AF3.1) |
| K0150-3 | anti-CDCP1 (CUB1) |
| K0150-4 | FITC labeled anti-CDCP1 (CUB1) |
| MK-13-3 | anti-Cdk2 (8A12) |
| K0065-3 | anti-Cdk4 (DCS-156) |
| K0066-3 | anti-Cdk6 (DCS-83) |
| K0067-3 | anti-Cdk6 (DCS-130) |
| K0068-3 | anti-Cdk7 (DCS-MO1) |
| K0077-3 | anti-$p16^{INK4a}$ (DCS-50) |
| M124-3 | anti-$p15^{INK4b}$ (1F6) |
| K0079-3 | anti-$p18^{INK4c}$ (DCS-118) |
| K0080-3 | anti-$p19^{INK4d}$ (DCS-100) |
| K0081-3 | anti-$p21^{WAF/CIP1}$ (DCS-60) |
| K0082-3 | anti-$p27^{Kip2}$ (DCS-72) |
| K0083-3 | anti-$p57^{Kip2}$ (DCS-230) |
| K0084-3 | anti-$p14^{ARF}$ (DCS-240) |
| K0085-3 | anti-Cdh1 (DCS-266) |
| K0086-3 | anti-Chk1 (DCS-310) |
| K0087-3 | anti-Chk2 (DCS-270) |
| K0088-3 | anti-Chk2 (DCS-273) |
| K0094-3 | anti-E2F-4 (TFE42) |
| K0095-3 | anti-DP-1 (TFD10) |
| M043-3 | anti-DJ-1 (3E8) |
| M069-3 | anti-MCM2 (4B8) |
| M038-3 | anti-MCM3 (3A2) |
| M049-3 | anti-MCM7 (4B4) |
| M050-3 | anti-RCC1 (3D11) |
| K0181-3 | anti-p53 (DO-1) |
| D241-3 | anti-phospho-p53 (Ser20) (17B6) |
| D240-3 | anti-phospho-p53 (Ser46) (#36) |
| CY-M1022 | anti-phospho-p53 Ser46 (TK-4D4) |
| D244-3 | anti-acetylated p53 (Lys120) (10E5) |
| K0059-3 | anti-phospho-p53 (Ser315) (FPS315) |
| D243-3 | anti-acetylated p53 (Lys382) (2B7E4) |
| K0060-3 | anti-phospho-p53 (Ser392) (FPS392) |
| D242-3 | anti-phospho p53 (Ser315) (#18) |
| CY-M1029 | anti-Acetylated Histone/p53-Lys382 (TM-5C5) |
| CY-7049 | CycLex® Total p53 ELISA Kit |
| CY-7050 | CycLex® Phospho-p53 Ser46 ELISA Kit |
| CY-7051 | CycLex® Phospho-p53 Ser392 ELISA Kit |
| D245-3 | anti-phospho c-Myc (Ser62) (33A12E10) |
| D246-3 | anti-phospho E2F1 (Ser364) (#2) |
| D247-3 | anti-phospho Mdmx (Ser367) (#15) |
| MK-15-1H | anti-Rb (3H9) |
| MK-15-3 | anti-Rb (3H9) |
| K0091-3 | anti-Rb2 (DCS-211) |
| M045-3 | anti-phospho Rb (Ser780) (2C4) |
| 555 | anti-phospho Rb (Ser780) (Polyclonal) |
| D248-3 | anti-phospho Rb (Ser795) (28B5) |
| D249-3 | anti phospho Rb (Thr821) (24A7) |
| D081-1 | anti-DNA Topoisomerase IIα (8D2) |
| M025-3 | anti-phospho DNA Topoisomerase IIα (3D4) |
| M052-3 | anti-DNA Topoisomerase II αβ (AK5) |
| M055-3 | anti-ORC2 (3B7) |
| M057-3 | anti-GAK (1C2) |
| M019-3 | anti-Nucleolin (4E2) |
| PM006-3 | anti-phospho Histone H3 (Polyclonal) |
| M123-3 | anti-ATR (4D7) |
| M131-3 | anti-ATM (4H1) |
| PM026 | anti-ATM (polyclonal) |