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

MONOCLONAL ANTIBODY

Anti-Human ORC2

Code No.	Clone	Subclass	Quantity	Concentration
M055-3	3B7	Mouse IgG1 κ	100 µL	1 mg/mL

BACKGROUND: In yeast *Saccharomyces cerevisiae*, DNA replication initiates from specific DNA sequences called autonomous replication sequences (ARSs), many of which have proven to be chromosomal origins of replication. A six-subunit protein complex named ORC (origin recognition complex) identified initially as ARS binding activity in an ATP-dependent manner. Because ORC remains bound to ARS throughout the cell cycle, initiation of replication, at least in *S. cerevisiae*, is not regulated by simply controlling the binding of ORC to origins but is thought to be due to the ORC dependent recruitment of other proteins such as MCMs onto the chromosome. Also, ORC may play a role in transcriptional "silencing" (Repression of transcription that appears to be due to a specialized chromatin structure) in *S. cerevisiae* mainly by recruiting the silencing apparatus. A silent chromatin share many properties of heterochromatin in *Drosophila* that is highly condensed state of chromatin where ORC might act in the formation or maintenance through interactions with heterochromatin-organizing factors. In higher eukaryotes, the replication origins have been more difficult to characterize at the molecular level. Recent study reported that the distance between chromatin-bound human ORC2 protein and chromatin-bound human MCM proteins must be at least 500-1,000 base pairs in HeLa cells. It is different from the case of yeast in which the distance between ORC and MCMs might be much smaller. ORC2 is a second largest subunit of ORC and its specific feature is largely remained unclear.

SOURCE: This antibody was purified from mouse ascites fluid using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse splenocyte immunized with full-length human ORC2 fusion protein (1-577 aa).

FORMULATION: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

SPECIFICITY: This antibody detects ~67 kDa of human ORC2 on Western blotting, Immunoprecipitation and Immunocytochemistry.

APPLICATIONS:

Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; 5 µg/600 µL of cell extract from $5x10^6$ cells
Immunohistochemistry; Not tested
Immunocytochemistry; 10 µg/mL
Flow cytometry; Not tested

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat	Hamster
Cells	Jurkat, Raji, HeLa	NIH/3T3, WR19L, P19	Rat-1	BHK
Reactivity on WB	+	-	-	-

INTENDED USE:

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13) Pak, D.T., *et al., Cell* **91**, 311-323 (1998)
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Clone 3B7 is used in the reference number 1)-7).

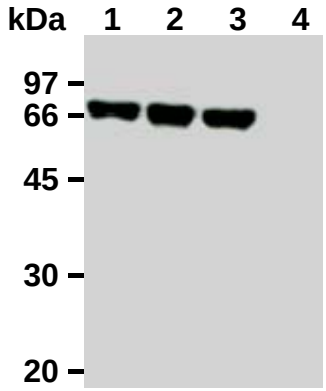
PROTOCOLS:

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 sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out 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 manufacturer'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 suggested 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, Raji, HeLa)

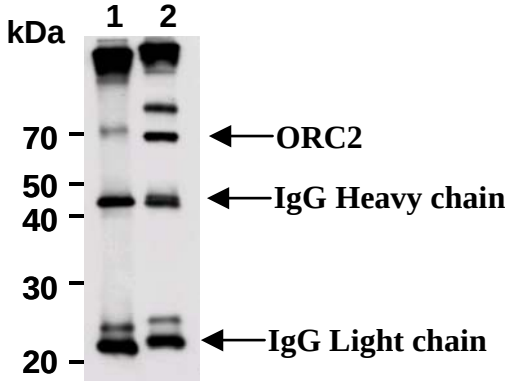


Western blot analysis of human ORC2 expression in Jurkat (1), Raji (2) HeLa (3) and NIH/3T3 (4) using M055-3.

Immunoprecipitation

1) Collect the cultured cells from 75-cm^2 flask (containing about 0.5-1 x 10^7 cells).
2) Wash the cells 2 times with PBS and suspend with 1,200 µL of cold Lysis buffer (50 mM HEPES-KOH, pH 7.5, 250 mM NaCl, 0.1% NP-40, 5 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).
3) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube.
4) Add 50 µL of 50% protein A agarose beads in the supernatant. Incubate it at 4°C with rotating for 60 minutes.
5) Centrifuge the tube at 12,000 x g for 5 minutes at 4°C. Supernatant is equally divided into another two tube.
6) Add the mouse IgG1 isotype control antibody (MBL; code no. M075-3) or anti-ORC2 antibody at the amount of as suggest in the **APPLICATIONS** to the supernatant. Vortex briefly and incubate with gently agitation for 30-120 minutes at 4°C.
7) Add 20 µL of 50% protein G agarose beads into the tube. Mix well and incubate with gentle agitation for 30-60 minutes at 4°C.
8) Wash the beads 3-5 times with ice-cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
9) Resuspend the beads in 30 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 15 µL/lane for the SDS-PAGE analysis.
(See **SDS-PAGE & Western blotting**.)

(Positive control for Immunoprecipitation; Jurkat)



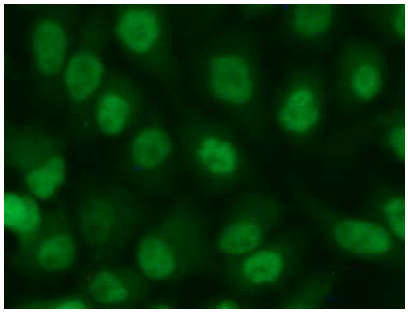
Immunoprecipitation of ORC2 from Jurkat with mouse IgG1 (1) or M055-3 (2). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with M055-3.*

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (for example, spread 5x10^4 cells for one slide, then incubate in a CO_2 incubator for one night.)
2) Wash the cells 3 times with PBS.
3) Fix the cells by immersing the slide in PBS containing 4% paraformaldehyde for 20 minutes at room temperature.

4) Wash the glass slide with PBS twice and once with PBS containing 0.05% Tween-20 (PBS-T).
5) Immerse the slide in PBS containing 0.1% TritonX-100 for 10 minutes at room temperature.
6) Wash the glass slide with PBS twice and once with PBS-T.
7) Add the primary antibody diluted with PBS as suggested 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.)
8) Wash the glass slide 3 times with PBS-T.
9) Add 100 μL of 1:100 FITC conjugated anti-mouse IgG (MBL; code no. IM-0819) diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
10) Wash the glass slide 3 times with PBS-T.
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)



Immunocytochemical detection of human ORC2 in HeLa with M055-3.

RELATED PRODUCTS:

M069-3	anti-MCM2 (4B8)
M038-3	anti-MCM3 (3A2)
M049-3	anti-MCM7 (4B4)
K0069-3	anti-CDC6 (DCS-180)
K0070-3	anti-CDC7 (DCS-342)
NH-12-3	anti-PCNA (5A10)
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)
K0162-3	anti-Cyclin A (E23.1)
K0163-3	anti-Cyclin A (E67.1)
K0163-6	anti-Cyclin A-biotin (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)
K0075-3	anti-CDC25C (DCS-193)
K0200-3	anti-Cdc25C (TC14)
CY-M1018	anti-Phospho-Cdc25C Ser216 (TK-1F1)
M123-3	anti-ATR (4D7)
M131-3	anti-ATM (4H1)
PM026	anti-ATM (polyclonal)
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)
K0081-3	anti-$p21^{WAF/CIP1}$ (DCS-60)

Other related antibodies and kits are also available.
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