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MONOCLONAL ANTIBODY

Anti-RCC1

Code No.	Clone	Subclass	Quantity	Concentration
M050-3	3D11	Mouse IgG1	100 µL	1 mg/mL

BACKGROUND: The human RCC1 (Regulator of Chromosome Condensation) gene has been isolated as a gene that rescues tsBN2 mutation. The rcc1$^-$ phenotype is pleiotropic and includes G_1 arrest, defects in mRNA splicing and mRNA transport. RCC1 is an abundant, highly conserved, chromatin-associated protein of 45 kDa which possesses an N-terminal region of 40 amino acid residues and an internal repeated domain in which about 60 amino acid residues are repeated seven times (RCC1 repeat). RCC1 is the principle mammalian guanine nucleotide exchange factor (GEF) for the nuclear G protein Ran, which GTPase activity is enhanced by GTPase-activating protein RanGAP located in the cytoplasm. RCC1 plays an important role in RNA transcription and processing, DNA replication, nuclear pore transport function and cell cycle regulation on Ran pathway.
Epitope of this antibody is N-terminal domain (13-21 aa) of RCC1.

SOURCE: This antibody was purified from hybridoma (clone 3D11) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse splenocyte immunized with the human full length RCC1.

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.

REACTIVITY: This antibody reacts with human, mouse and rat RCC1 (45 kDa) on Western blotting, Immunoprecipitation and Immunocytochemistry. This antibody does not inhibit GEF activity of RCC1.

APPLICATIONS:
Western blotting; 1 µg/mL
Immunoprecipitation; 2 µg/200 µL of cell extract from 5 x 10^6 cells
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, U937, HeLa, HL60, HepG2, ZR75-1, MRC-5, Molt4, A431	WR19L, Ba/F3, NIH/3T3	P19, Rat1
Reactivity on WB	+	+	+

INTENDED USE:
For Research Use Only. Not for use in diagnostic procedures.

REFERENCES:
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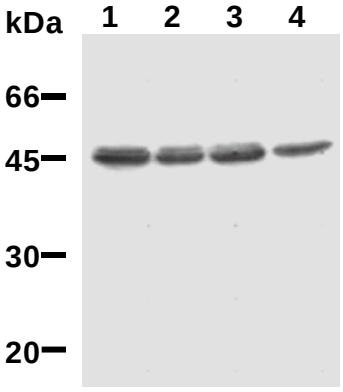
2) Li, H. Y., *et al*., *J. Cell Biol*. **160**, 635-644 (2003)
3) Azuma, Y., *et al., J. Biochem.* **122**, 1133-1138 (1997)
4) Lounsbury, S., *et al., J. Biol. Chem.* **272**, 551-555 (1997)
5) Richards, S., *et al., Science* **276**, 1842-1844 (1997)
6) Seki, T., *et al., J. Biochem.* **120**, 207-214 (1996)
7) Noguchi, S., *et al., EMBO J*. **15**, 5595-5605 (1996)
8) Saitoh, H., *et al., J. Biol. Chem*. **270**, 10658-10663 (1995)
9) Coutavas, E., *et al., Nature* **366**, 585-587 (1993)

Clone 3D11 is used in reference number 1) and 2).



Western blotting analysis of RCC1 expression in Jurkat (1), HeLa (2), Raji (3) and WR19L (4) using M050-3.

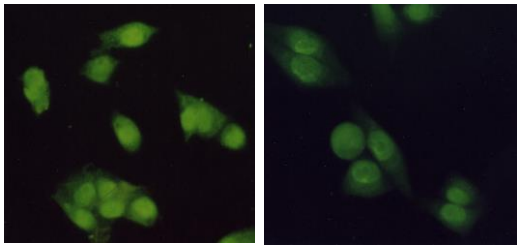
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

PROTOCOLS:

SDS-PAGE & Western blotting

1) Wash cells (approximately 1 x 10^7 cells) 3 times with PBS and resuspend them in 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 protease inhibitors at appropriate concentrations. Incubate it at 4ºC with rotating for 30 minutes; thereafter, briefly sonicate the mixture (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4ºC and transfer the supernatant to another fresh tube. Measure the protein concentration of the supernatant and add the cold 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% methanol). 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 4ºC.
7) Incubate the membrane for 1 hour at room temperature with primary antibody diluted with PBS (pH 7.2) containing 1% skimmed milk as suggested in the **APPLICATIONS**. (The concentration of antibody will depend on the conditions.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3).
9) Incubate the membrane with 1:10,000 Anti-IgG (H+L chain) (Mouse) pAb-HRP (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 (10 minutes x 3).
11) Wipe excess buffer off the membrane, and incubate membrane with an appropriate chemiluminescence reagent for 1 minute.
12) Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
13) Expose the membrane onto an X-ray film in a dark room for 3 minutes.
14) Develop the film under usual settings. The conditions for exposure and development may vary.

(Positive controls for Western blotting; Jurkat, Raji, HeLa, WR19L)

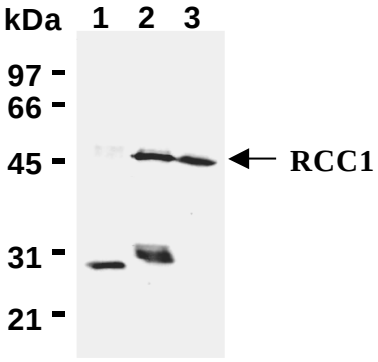


Immunocytochemical detection of RCC1 on 4% paraformaldehyde fixed HepG2 cells (left) and HeLa cells (right) with M050-3.

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (for example, spread 1x10^4 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 3 times with PBS.
5) Immerse the slide in PBS containing 0.1% Triton X-100 for 10 minutes at room temperature.
6) Wash the glass slide 3 times with PBS.
7) Add the primary antibody diluted with PBS as suggested in the **APPLICATIONS** onto the cells and incubate for 30 minutes at room temperature. (Optimization of antibody concentration or incubation condition are recommended if necessary.)
8) Wash the glass slide 3 times with PBS.
9) Add 100 μL of 1:100 FITC conjugated anti-mouse IgG (Beckman coulter; code no. IM0819) 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.
11) Wipe excess liquid off the glass 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 controls for Immunocytochemistry; HepG2, HeLa)



Immunoprecipitation of RCC1 from HeLa with mouse IgG1 (1) or M050-3 (2). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with M050-3.*

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 protease inhibitors at appropriate concentrations. Incubate at 4ºC with rotation for 30 minutes; thereafter, briefly sonicate the mixture (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4ºC and transfer the supernatant to another tube.

3) Add primary antibody as suggested in the **APPLICATIONS** into 200 µL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4ºC. Add 20 µL of 50% protein A agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4ºC.
4) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
5) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10 µL/lane for the SDS-PAGE analysis.
(See **SDS-PAGE & Western blotting**.)

(Positive control for Immunoprecipitation; HeLa)

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