OriGene Technologies, Inc.
9620 Medical Center Drive, Ste 200
Rockville, MD 20850, US
Phone: +1-888-267-4436
https://www.origene.com
techsupport@origene.com
EU: info-de@origene.com
CN: techsupport@origene.cn

Product datasheet for AM26601AF-N

RCC1 (13-21) Mouse Monoclonal Antibody [Clone ID: 3D11]

Product data:

Product Type:	Primary Antibodies
Clone Name:	3D11
Recommended Dilution:	**Western blot:** 1 µg/ml for a chemiluminescence detection system. **Immunocytochemistry:** 5µg/ml. **Immunoprecipitation:** 2 µg/200 µl of cell extract from 5 x 10^6 cells.
Reactivity:	Human, Mouse, Rat
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	Full-length human RCC1
Specificity:	This antibody detects N-terminal domain (13-21 aa) of RCC1. It does not inhibit GEF activity of RCC1.
Formulation:	PBS (pH 7.2) / 1% sucrose. Contains no preservative. State: Azide Free State: Lyophilized Ig fraction
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 (in aliquots) at -20°C. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Gene Name:	regulator of chromosome condensation 1
Database Link:	Entrez Gene 1104 Human P18754

View online »

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-associat ed protein of 45 kDa which possesses an N-terminal region of 40 amino acid residues and an internal repeated doma in 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.

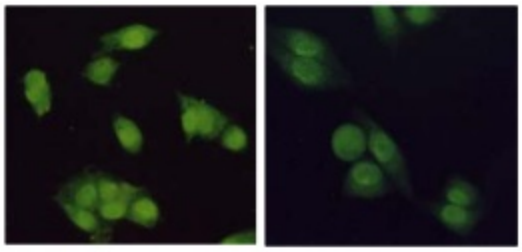
Synonyms: RCC1-I

Note: This product was originally produced by MBL International.

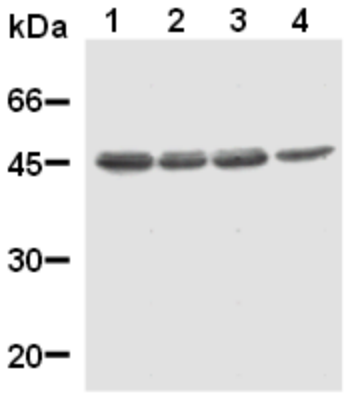
Protocol:
SDS-PAGE & Western Blotting
1) Wash cells (approximately 1 x 10 7 cells) 3 times with PBS and resuspend them in 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing protease inhibitors at appropriate concentrations. Incubate it at 4 o C 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 4 o C and transfer the supernatant to an other 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 µ L 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 o C.
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 times).
9) Incubate the membrane with 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 (10 minutes x 3 times).
11) Wipe excess buffer off the membrane, and incubate membrane with an appropri ate 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)
Immunocytochemistry
1) Culture the cells in the appr opriate condition on a glass slide. (for exam ple, 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 dilu ted 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 µ L of 1:100 FITC conjugated anti-mouse IgG 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 Immunoc ytochemistry; HepG2, HeLa)
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 4 o C 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 4 o C and transfer the supernatant to another tube.
3) Add primary antibody as suggested in the APPLICATIONS into 200 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 cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4 o C.
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 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 control for Immunoprecipitation; HeLa)

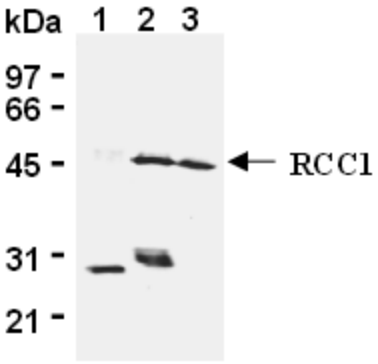
Product images:



Immunocytochemical detection of RCC1 on 4% paraformaldehyde fixed HepG2 cells (left) and HeLa cells (right) with AM26601AF-N.



Western blot analysis of RCC1 expression in Jurkat (1), HeLa (2), Raji (3) and WR19L (4) using AM26601AF-N.



Immunoprecipitation of RCC1 from HeLa with mouse IgG1 (1) or AM26601AF-N (2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with AM26601AF-N.