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Product datasheet for AM26604AF-N

ORC2 (1-577) Mouse Monoclonal Antibody [Clone ID: 3B7]

Product data:

Product Type:	Primary Antibodies
Clone Name:	3B7
Applications:	IF, IP, WB
Recommended Dilution:	**Western blot:** 1 µg/ml for chemiluminescence detection system. **Immunoprecipitation:** 5 µg/600 µl of cell extract from 5x 10^6 cells. **Immunocytochemistry:** 10 µg/ml.
Reactivity:	Human
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	Full-length human ORC2 fusion protein (1-577 aa)
Specificity:	This antibody detects ~67 kDa of ORC2.
Formulation:	PBS containing 50% glycerol, pH 7.2. Contains no preservative. State: Azide Free State: Liquid Ig fraction
Concentration:	lot specific
Purification:	Protein A agarose
Conjugation:	Unconjugated
Storage:	Upon receipt, store undiluted (in aliquots) at -20°C. Avoid repeated freezing and thawing.
Stability:	Shelf life: One year from despatch.
Gene Name:	origin recognition complex subunit 2
Database Link:	Entrez Gene 4999 Human Q13416

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Background: The origin recognition complex (ORC) is a highly conserved six subunits protein complex essential for the initiation of the DNA replication in eukaryotic cells. Studies in yeast demonstrated that ORC binds specifically to origins of replication and serves as a platform for the assembly of additional initiation factors such as Cdc6 and Mcm proteins. ORC2L is a subunit of the ORC complex. ORC2L forms a core complex with ORC3L, 4L, and 5L. It also interacts with CDC45L and MCM10, which are proteins known to be important for the initiation of DNA replication. ORC2L specifically associate with the origin of replication of Epstein Barr virus in human cells, and is thought to be required for DNA replication from viral origin of replication.

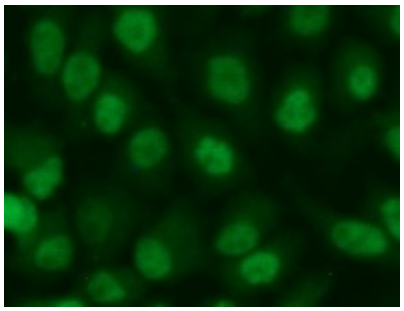
Synonyms: RRR1, SIR5, YBR060C, YBR0523, ORC2L

Note: This product was originally produced by MBL International.

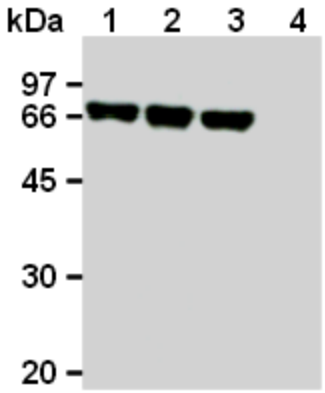
Protocol:
SDS - PAGE & Western Blotting
1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris - HCl, pH 7.2, 250 mM NaCl, 0.1% NP - 40, 2 mM EDTA, 10% glycerol) co ntaining appropriate protease inhibitors. Incubate it at 4 o C with rotating for 30 minutes, then 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 c oncentration 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 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 with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as s uggest ed 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 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)
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 µ L 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 o C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
3) Centrifuge the tube a t 12,000 x g for 10 minutes at 4 o C and transfer the supernatant to another tube.
4) Add 50 µ L of 50% protein A agarose beads in the supernatant. Incubate it at 4 o C with rotating for 60 minutes.
5) Centrifuge the tube at 12,000 x g for 5 minutes at 4 o C. Supernat ant is equally divided into another two tube.
6) Add the mouse IgG1 isotype control antibody 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 o C .
7) Add 20 µ L of 50% protein G agarose beads into the tube. Mix well and incubate with gentle agitation for 30 - 60 minutes at 4 o 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 µ L of Laemmli' s sample buffer, boil for 3 - 5 minutes, and centrifuge for 5 minutes. Use 15 µ L/lane for the SDS - PAGE analysis. (See SDS - PAGE & Western blotting.)
(Positive control for Immunoprecipitation; Jurkat)

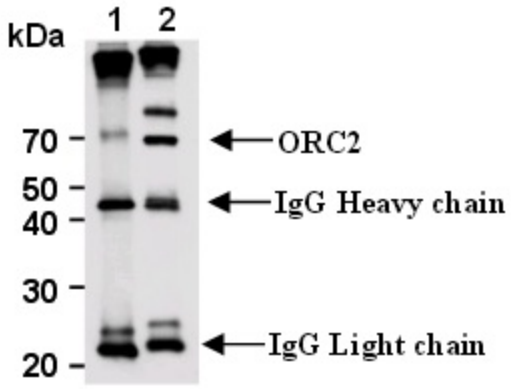
Product images:



Immunocytochemical detection of human ORC2 in HeLa with AM26604AF-N



Western blot analysis of human ORC2 expression in Jurkat (1), Raji (2) HeLa (3) and NIH/3T3 (4) using AM26604AF-N.



Immunoprecipitation of ORC2 from Jurkat with mouse IgG1 (1) or AM26604AF-N (2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS - PAGE and immunoblotted with AM26604AF-N.