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 AM26485AF-N

RNF2 Mouse Monoclonal Antibody [Clone ID: 3-3]

Product data:

Product Type:	Primary Antibodies
Clone Name:	3-3
Applications:	IP, WB
Recommended Dilution:	Western blot: 1 µg/m. Immunoprecipitation: 1-5 µg / 200-300 µl of cell extract. For details see protocols below. Not recommended for Immunohistochemistry.
Reactivity:	Hamster, Human, Mouse
Host:	Mouse
Isotype:	IgG2b
Clonality:	Monoclonal
Immunogen:	GST fusion mouse full-length Ring1B protein
Specificity:	This antibody reacts with Ring1B.
Formulation:	PBS containing 50% glycerol, pH 7.2. No preservative is contained. State: Azide Free State: Liquid Ig fraction
Concentration:	lot specific
Purification:	Protein-A Sepharose
Conjugation:	Unconjugated
Storage:	Store undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Gene Name:	ring finger protein 2
Database Link:	Entrez Gene 6045 Human Q99496

View online »

Background: Polycomb-group (PcG) proteins form multimeric complexes that maintain the state of transcriptional repression of several regulatory genes during development. Ring1B/Rnf2 forms part of a protein complex containing other PcG proteins, such as Mel18, Bmi1, MBLR, MPc3, and the spliceosome protein Sap155, and these complexes associate with chromatin to regulate transcription. Ring1B may also play a role in the regulation of Hox gene expression by PcG complexes. Deletion of Ring1B activity results in gastrulation arrest and cell cycle inhibition.

Synonyms: RING finger protein 2, BAP1, BAP-1, DING, HIPI3, RING1B

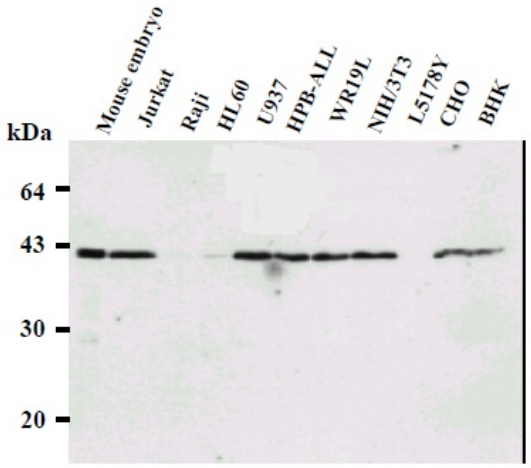
Note: This product was originally produced by MBL International.

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) containing appropriate protease inhibitors. Incubate it at 4 oC 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 oC 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/cm2 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 the anti-Ring1B monoclonal antibody (1 µg/mL) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
8) Wash the membrane with PBS (5 minutes x 6 times).
9) Incubate the membrane with the 1:10000 POD-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 (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, NIH/3T3, CHO, BHK

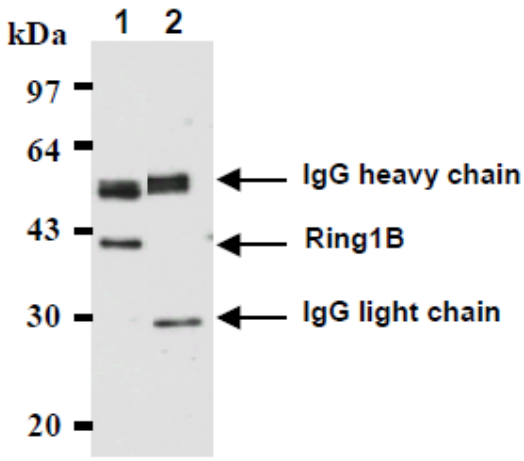
Immunoprecipitation
1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM

Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4 oC 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 oC and transfer the supernatant to another tube.
3) Add 1-5 µg of the anti-Ring1B monoclonal antibody into 250 µL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4 oC. Add 20 µL of 50% Protein A-agarose beads resuspended in the Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4 oC.
4) Wash the beads 3-5 times with ice-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: U937

Product images:



Western blot analysis of Ring1B expression in several cells using AM26485AF-N.



Immunoprecipitation of Ring1B from U937 cells with AM26485AF-N (1) or normal mouse IgG (2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with AM26485AF-N.