

Product datasheet for AM26485AF-N

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

Product data:

OriGene Technologies, Inc.

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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
lsotype:	lgG2b
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 lg 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:	<u>Entrez Gene 6045 Human</u> <u>Q99496</u>



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	RNF2 Mouse Monoclonal Antibody [Clone ID: 3-3] – AM26485AF-N
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-HCI, 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 Laemmll's sample buffer. 4) Boil the samples for 2 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 m 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. 6) 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 dabing 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

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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 μ g of the anti-Ring1B monoclonal antibody into 250 μ L of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4 oC. Add 20 μ L 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 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: U937

Product images:



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

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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.

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