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MONOCLONAL ANTIBODY

Anti-Drebrin mAb

Code No.	Clone	Subclass	Quantity	Concentration
D029-3	M2F6	Mouse IgG1 κ	100 µL	1 mg/mL

BACKGROUND: In developing neuronal tissue, various functional molecules appear timingly and influence the processes of cell differentiation, migration, neurite outgrowth, and synaptic formation. Drebrins (developmentally regulated brain proteins) are candidates of such functional molecules, because they were expressed peculiarly in the course of development of neuronal tissues. Drebrin proteins are classified into an embryonic form (E) and an adult form (A) in mammals. The embryonic form is further classified into E1 and E2 in the chicken.

SOURCE: This antibody was purified from hybridoma (clone M2F6) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell X-63-Ag-8-653 with Balb/c mouse splenocyte immunized with purified chicken drebrin E.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

FORMULATION: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

REACTIVITY: This antibody reacts with Drebrin E and Drebrin A on Western blotting, Immunoprecipitation, Immunohistochemistry and Immunocytochemistry.

APPLICATIONS:

Western blotting; 1 µg/mL
Immunoprecipitation; 5 µg/2.5 x 10^6 cells
Immunohistochemistry; Reference 14)
Heat treatment is necessary for paraffin embedded sections.
Microwave oven; 2 times for 10 minutes each in 10 mM citrate buffer (pH 6.5)
Immunocytochemistry; 1 µg/mL
Flow cytometry; Not tested

Detailed procedure is provided in the following **PROTOCOLS**.

INTENDED USE:

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SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat	Bovine	Chicken	Cat	Rabbit
Cells or Tissues	Jurkat, HeLa	NIH/3T3, Brain	PC12, Brain	kidney cortices*	Brain**	visual cortex†	Gastic mucosa‡
Reactivity on WB	+	+	+	+	+	+	+

It is reported in the reference number *6) **14) †13) ‡4)

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9) Hayashi, K., and Shirao, T., *J. Neurosci.* **19**, 3918-3925 (1999) [IC]
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12) Asada, H., *et al., J. Neurosci. Res.* **38**, 149-159 (1994)
13) Imamura, K., *et al., Neurosci. Res.* **13**, 33-41 (1992) [WB, IHC]
14) Shirao, T., *et al., Dev. Brain Res.* **29**, 233-244 (1986) [WB, IHC]

Clone M2F6 is used in these references.
For more information, please visit our website at http://ruo.mbl.co.jp/.

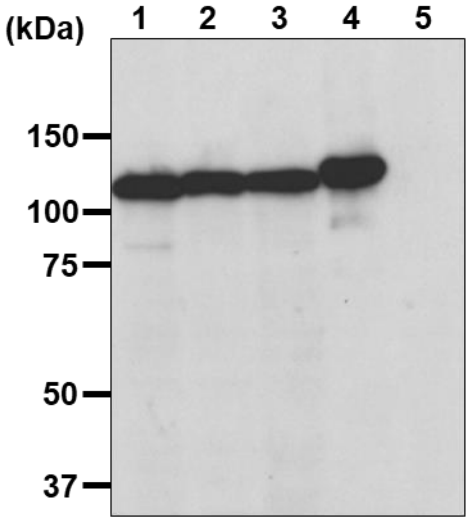
PROTOCOLS:

SDS-PAGE & Western blotting

1) Wash 1 x 10^7 cells 3 times with PBS and suspend them in 1 mL of Laemmli's sample buffer, then sonicate briefly (up to 20 seconds).
2) Boil the samples for 3 minutes and centrifuge. Load 10 µL of sample per lane on a 1-mm-thick SDS-polyacrylamide gel (7.5% acrylamide) and carry out electrophoresis.
3) 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.

4) 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.
5) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
7) Incubate the membrane with the 1:10,000 anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
8) Wash the membrane with PBS-T (5 minutes x 3 times).
9) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
10) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
11) Expose to an X-ray film in a dark room for 2 minutes.
12) Develop the film as usual. The condition for exposure and development may vary.

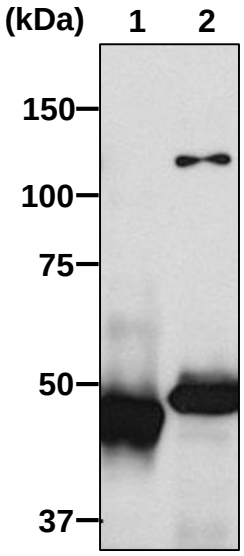
(Positive controls for Western blotting; HeLa, NIH/3T3, PC12, rat brain)



Western blotting analysis of Drebrin expression in HeLa (1), NIH/3T3 (2), PC12 (3), rat brain (4) and L (5) using D029-3.

Immunoprecipitation

1) Wash 1 x 10^7 cells 3 times with PBS and suspend with 1 mL of cold Extraction buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 0.05% NP-40) containing appropriate protease inhibitors, then sonicate briefly (up to 15 seconds).
2) Incubate the tube for 10 minutes on ice.
3) Centrifuge the tube at 12,000 x g for 5 minutes at 4°C and transfer the supernatant to another fresh tube.
4) Mix 20 µL of 50% protein A agarose beads slurry resuspended in 500 µL of IP buffer (50 mM Tris-HCl pH 8.8, 3 M NaCl) with primary antibody as suggested in the **APPLICATIONS**. Incubate with gentle agitation for 1 hour at room temperature.
5) Centrifuge the tube at 2,000 x g for 10 seconds and discard the supernatant.
6) Resuspend the beads with 1 mL of IP buffer.
7) Centrifuge the tube at 2,000 x g for 10 seconds and discard the supernatant.
8) Repeat steps 6)-7) 3 times.
9) Add 250 µL of cell lysate (prepared sample from step 3)), then incubate with gentle agitation for 1 hour at room temperature.
10) Centrifuge the tube at 2,000 x g for 10 seconds and discard the supernatant.
11) Resuspend the beads with 1 mL of Extraction buffer.
12) Centrifuge the tube at 2,000 x g for 10 seconds and discard the supernatant.
13) Repeat steps 11)-12) 6 times.
14) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3 minutes, and centrifuge for 5 minutes. Use 10 µL/lane for the SDS-PAGE analysis.
(See **SDS-PAGE & Western blotting**.)



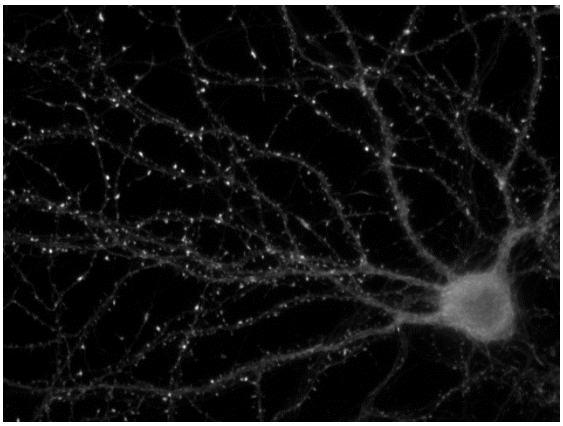
Immunoprecipitation of Drebrin from HeLa with or Mouse IgG1(1) or D029-3 (2). *After immunoprecipitation with the antibody, Immunocomplex was resolved on SDS-PAGE and immunobloted with D029-3.*

(Positive control for Immunoprecipitation; HeLa)

Immunocytochemistry

1) Spread the cells on a glass slide, then incubate in a CO_2 incubator for one night.
2) Remove the culture supernatant by careful aspiration.
3) Wash the slide 2 times with PBS.
4) Fix the cells with 4% paraformaldehyde (PFA)/PBS for 20 min. at room temperature (20~25°C).
5) Wash the slide 3 times with PBS.
6) Add 3% BSA/PBS to the cell and incubate for 1 hour at room temperature.
7) Tip off 3% BSA/PBS and add 200 µL of the primary antibody diluted with PBS as suggested in the **APPLICATIONS** onto the cells. Incubate for 16 hours at 4°C. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
8) Wash the slide 3 times in PBS for 5 minutes each.
9) Add 200 µL of 1:100 FITC conjugated anti-mouse IgG (Cappel; code no. 55514) diluted with PBS onto the cells. Incubate for 1 hour at room temperature. Keep out light by aluminum foil.
10) Wash the slide 3 times in PBS for 5 minutes each.
11) Wipe excess liquid from the 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 control for Immunocytochemistry; Primary cultured hippocampal neuronal cell (21DIV))



Immunocytochemical detection of Drebrin on primary cultured hippocampal neuronal cell (21DIV).
Data was provided by Drs. Tomoaki Shirao and Hiroyuki Yamazaki (Department of Neurobiology and Behavior, Gunma University Graduate School of Medicine)

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