**For Research Use Only.
Not for use in diagnostic procedures.**

MONOCLONAL ANTIBODY

Anti-Borealin (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
M147-3	1D11	Mouse IgG2a	100 µL	1 mg/mL

BACKGROUND: Borealin is a subunit of the chromosomal passenger complex (CPC), which is necessary for chromosome segregation and cytokinesis. While CPC shows a dynamic localization pattern during mitosis, Borealin and survivin, another subunit of CPC is essential to localize CPC at centromare from prophase to anaphase. It is thought that Borealin and survivin recruit aurora B, a kinase subunit of CPC, to inner centromare to allow phosphorylating appropriate substrates.

SOURCE: This antibody was purified from hybridoma (clone 1D11) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with C3H mouse lymphocyte immunized with the recombinant full-length human Borealin (1-280 aa).

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

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

REACTIVITY: This antibody reacts with Borealin (35 kDa) on Western blotting, Immunoprecipitation and Immunocytochemistry.

APPLICATIONS:

Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; 1 µg/50 µL of cell extract from 1 x 10^6 cells
Immunohistochemistry; Not tested
Immunocytochemistry; 1-5 µg/mL
Flow cytometry; Not tested

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

SPECIES CROSS REACTIVITY:

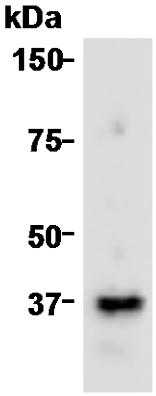
Species	Human	Mouse	Rat
Cell	293T	Not tested	Not tested
Reactivity on WB	+		

INTENDED USE:
For Research Use Only. Not for use in diagnostic procedures.

REFERENCES:

1) Weiderhold, K. N., *et al.*, *PLoS One* **11**, e0153455 (2016) [IC]
2) Labarrade, F., *et al.*, *Int. J. Cosmet. Sci.* (2016) In press.
3) Klein, U. R., *et al.*, *Mol. Biol. Cell* **17**, 2547-2558 (2006)
4) Gassmann, R., *et al.*, *J. Cell Biol*. **166**, 179-191 (2004)
5) Sampath, S. C., *et al.*, *Cell* **118**, 187-202 (2004)
6) Walker, M.G., *Curr. Cancer Drug Targets* **1**, 73-83 (2001)

This antibody has been used in the reference number 1)-2).



Western blot analysis of Borealin expression in 293T using M147-3.

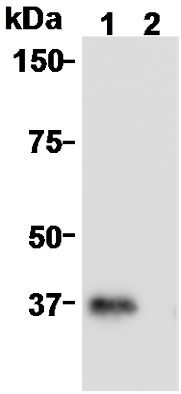
PROTOCOLS:

SDS-PAGE & Western Blotting

1) Wash the 5x10^6 cells 3 times with PBS and suspend with 500 µL of Laemmli's sample buffer.
2) Boil the samples for 2 minutes and centrifuge. Load 5 µL of sample per lane on a 1-mm-thick SDS-polyacrylamide gel 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% MeOH). See the manufacturer's manual for precise transfer procedure.
4) To reduce nonspecific binding, soak the membrane in 5% 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 PBS, pH 7.2 containing 1% BSA, 0.1% Tween-20 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 (5 minutes x 3 times).
7) Incubate the membrane with 1:10,000 of Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% BSA (in PBS, pH 7.2) for 1 hour at room temperature.
8) Wash the membrane with PBS (5-15 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 control for Western blotting; 293T)

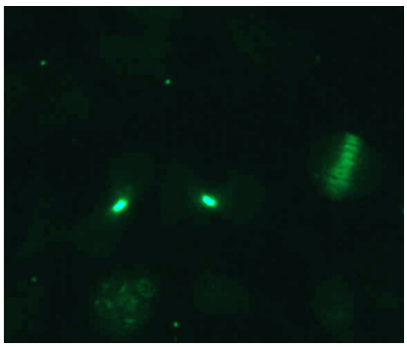


Immunoprecipitation of Borealin from 293T with M147-3 (1) or normal mouse IgG (2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with M147-3.

Immunoprecipitation

1) Wash the cells 3 times with PBS and suspend with 250 µL of cold Lysis buffer [10 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1% sodium deoxycholate, 1% NP-40, 0.1% SDS] containing appropriate protease inhibitors. Incubate it at 4°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°C and transfer the supernatant to another tube.
3) Add primary antibody as suggested in the **APPLICATIONS** into 50 µL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C.
4) Add 10 µL of 50% protein A agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
5) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
6) Resuspend the beads in 10 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.
7) 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.
8) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
9) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% BSA, 0.1% Tween-20 as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
10) Wash the membrane with PBS (5 minutes x 3 times).
11) Incubate the membrane with the 1:5,000 Mouse TrueBlot HRP-conjugated anti-mouse IgG (eBiosceince; code no. 18-8877-33) diluted with 1% BSA (in PBS, pH 7.2) for 1 hour at room temperature.
12) Wash the membrane with PBS (5-15 minutes x 3 times).
13) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
14) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
15) Expose to an X-ray film in a dark room for 2 minutes.
16) Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; 293T)



Immunocytochemical detection of Borealin on acetone fixed HEp-2 cells with M147-3.

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (for example, spread 1 x 10^4 cells per one well, then incubate in a CO_2 incubator for one night.)
2) Fix the cells by immersing the slide in Acetone for 10 minutes on ice.
3) Prepare a wash container such as a 500 mL beaker with a magnetic stirrer. Then wash the cultured cells on the glass slide by soaking the slide with a plenty of PBS in the wash container for 5 minutes. Take care not to touch the cells. Repeat another wash once more.
4) Immerse the slide in PBS containing 0.1% Triton X-100 for 5 minutes at room temperature.
5) Wash the slide in a plenty of PBS as in the step 3).

6) Add the primary antibody diluted with PBS as suggested in the **APPLICATIONS** onto the cells and incubate for 1 hour at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
7) Wash the slide in a plenty of PBS as in the step 3).
8) Add 30 μL of FITC conjugated anti-mouse IgG antibody diluted with PBS onto the cells. Incubate for 1 hour at room temperature. Keep out light by aluminum foil.
9) Wash the slide in a plenty of PBS as in the step 3).
10) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry. Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; HEp-2)

RELATED PRODUCTS:

CY-1174	CycLex® Aurora Family Kinase Assay /Inhibitor Screening Kit
M076-3	Mouse IgG2a (isotype control) (6H3)