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

MONOCLONAL ANTIBODY

Anti-Human Nectin-3/CD113

Code No.	Clone	Subclass	Quantity	Concentration
K0224-3	N3.12.4	Mouse IgG2a κ	100 μg	1 mg/mL

BACKGROUND: Nectins originally identified as poliovirus receptor-related protein (PRR) are Ca^{2+}-independent immunoglobulin-like cell-cell-adhesion molecules. Nectins comprise a family of four members: nectin-1 (CD111/PRR1), nectin-2 (CD112/PRR2), nectin-3 (CD113/PRR3) and nectin-4 (PRR4). Nectin-2 and -3 are ubiquitously expressed whereas nectin-1 is abundantly expressed in brain, and nectin-4 is in placenta. Nectins colocalized with afadin, which an actin-filament binding protein to associated with the actin cytoskeleton. Nectins play important roles in diverse cell-cell junctions.

SOURCE: This antibody was purified from hybridoma (clone N3.12.4) supernatant using protein A agarose. The mouse was immunized with recombinant human Nectin-3 Fc fusion protein.

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 V domain of human Nectin-3 on Immunocytochemistry and Flow cytometry.

APPLICATIONS:

Western blotting; Not recommended
Immunoprecipitation; 5 μg/200 μL of cell extract from 5 x 10^6 cells
Immunohistochemistry (paraffin section); Not recommended
Immunocytochemistry; 10 μg/mL
Flow cytometry; 10 μg/mL (final concentration)

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	U251	Not Tested	Not Tested
Reactivity on IC	+		

INTENDED USE:
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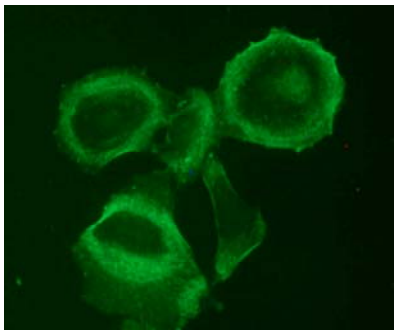
REFERENCE:
1) Takai, Y., and Nakanishi, H., *J. Cell Sci.* **116**, 17-27 (2003)



Immunocytochemical detection of Nectin-3 on U251 with K0224-3.

PROTOCOLS:

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (for example, spread 1x10^4 cells for one slide, then incubate in a CO_2 incubator for one night.)
2) Wash the cells 3 times with PBS.
3) Fix the cells by immersing the slide in PBS containing 4% paraformaldehyde (PFA) for 15 minutes at room temperature.
4) Wash the glass slide 2 times with PBS.
5) Add 50 μL of Clear Back (human Fc receptor blocking reagent, MBL; code no. MTG-001) to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
6) Add the primary antibody diluted with PBS as suggested in the **APPLICATIONS** onto the cells and incubate for 30 minutes at room temperature. (Optimization of antibody concentration or incubation condition are recommended if necessary.)
7) Wash the glass slide 2 times with PBS.
8) Add 100 μL of 1:100 FITC conjugated anti-mouse IgG (MBL; code no. IM-0819) diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
9) Wash the glass slide 3 times with PBS.
10) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.

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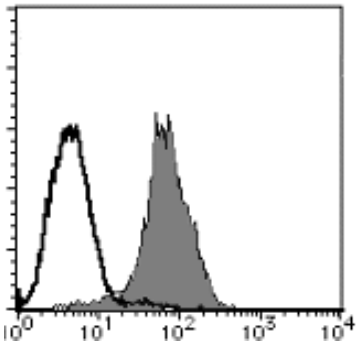
URL https://ruo.mbl.co.jp
e-mail support@mbl.co.jp, TEL 052-238-1904

11) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; U251)



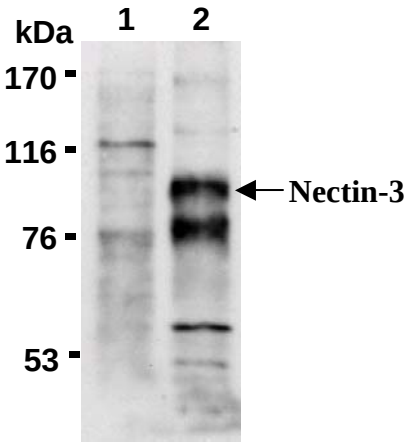
Flow cytometric analysis of human Nectin-3 expression on U251. *Open histogram indicates the reaction of isotypic control to the cells. Shaded histogram indicates the reaction of K0224-3 to the cells.*

Flow cytometric analysis for floating cells

We usually use Fisher tubes or equivalents as reaction tubes for all steps described below.

1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN_3].
2) Resuspend the cells with washing buffer ($5x10^6$ cells/mL).
3) Add 50 µL of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature (20~25°C). Remove supernatant by careful aspiration.
4) Add 20 µL of Clear Back (human Fc receptor blocking reagent, MBL; code no. MTG-001) to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
5) Add 40 µL of the primary antibody at the concentration as suggested in the **APPLICATIONS** diluted in the washing buffer. Mix well and incubate for 30 minutes at room temperature.
6) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
7) Add 20 µL of 1:100 FITC conjugated anti-mouse IgG (MBL; code no. IM-0819) diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
8) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
9) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; U251)



Immunoprecipitation of Nectin-3 from biotinylated U251 with Mouse IgG2a (1) or K0224-3 (2). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with HRP labeled streptavidin (MBL; code no. IM-0309).*

Immunoprecipitation

1) Wash biotinylated cells (approximately 1 x 10^7 cells) 3 times with PBS and resuspend them in 1 mL of cold Lysis buffer (50 mM Tris-HCl pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing protease inhibitors at appropriate concentrations. Incubate it at 4°C with rotating for 15 minutes.
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another fresh tube.
3) Add 50 µL of 50% protein A agarose beads in the supernatant. Incubate it at 4°C with rotating for 60 minutes.
4) Centrifuge the tube at 12,000 x g for 5 minutes at 4°C. Supernatant is equally divided into another fresh two tube.
5) Add the mouse IgG2a isotype control antibody (MBL; code no. M076-3) or anti-Nectin-3 antibody at the amount of as suggested in the **APPLICATIONS** to the supernatant. Vortex briefly and incubate with gently agitation for 60-120 minutes at 4°C.
6) Add 20 µL of 50% protein A agarose beads into the tube. Mix well and incubate with gentle agitation for 30-60 minutes at 4°C.
7) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
8) Resuspend the beads in 30 µL of Laemmli's sample buffer and boil the samples for 2 minutes and centrifuge. Load 15 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
9) 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.
10) 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.
11) Wash the membrane with PBS-T [0.05% Tween-20 in

PBS] (5 minutes x 3 times).

12) Incubate the membrane with 1:10,000 HRP-conjugated streptavidin (MBL; code no. IM-0309) diluted with PBS-T for 5-30 minutes at room temperature.
13) Wash the membrane with PBS-T (5 minutes x 3 times).
14) Wipe excess buffer off the membrane, and incubate membrane with an appropriate chemiluminescence reagent for 1 minute.
15) Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
16) Expose the membrane onto an X-ray film in a dark room for 1 minutes. Develop the film under usual settings. The conditions for exposure and development may vary.

(Positive control for Immunoprecipitation; U251)

RELATED PRODUCTS:

K0224-A48	Alexa Fluor® 488 labeled anti-human Nectin-3 (N3.12.4)
D175-3	CD112/Nectin-2 (TX31)
D175-4	FITC labeled CD112/Nectin-2 (TX31)
D146-3	anti-mouse Nectin-1 (48-12)
D083-3	Mouse CD112/Nectin-2 (502-57)
D084-3	Mouse CD113/Nectin-3 (103-A1)
M076-3	Mouse IgG2a isotype control (6H3)