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

MONOCLONAL ANTIBODY

Anti-Human Nestin

Code No.	Clone	Subclass	Quantity	Concentration
K0199-3	10C2	Mouse IgG1	100 µg	1 mg/mL

BACKGROUND: Nestin is a large (220-240 kDa) Class VI intermediate filament that is abundantly expressed in the developing central nervous system (CNS) in early embryonic neuroepithelial stem cells. Upon terminal neural differentiation, nestin is down regulated and replaced by neurofilaments. Since nestin is absent from nearly all mature CNS cells, it is commonly used as a marker for neuronal stem cells. Nestin is also expressed in gliomas, melanoma, and tumor endothelial cells, making it an excellent angiogenic marker to evaluate neovascularity of endothelial cells in tumors.

SOURCE: This antibody was purified from hybridoma (clone 10C2) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3-X63-Ag8-653 with Balb/c mouse splenocyte immunized with full-length human recombinant Nestin.

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 human Nestin (220-240 kDa) on Western blotting.

APPLICATIONS:

Western blotting; 1 µg/mL for chemiluminescence detection system
Immunohistochemistry; 10 µg/mL
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; Not tested
Immunoprecipitation; Not recommended
Flow cytometry; 10 µg/mL (final concentration)

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

INTENDED USE:
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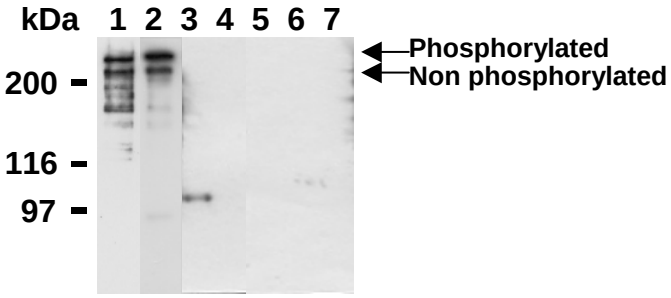
SPECIES CROSS REACTIVITY:

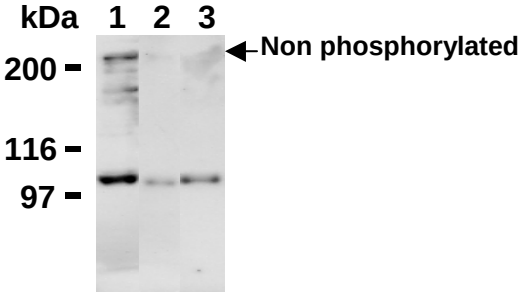
Species	Human		Mouse	Rat
Cells	U251, SK-N-SH, U87/MG	Jurkat, Raji, HeLa, ZR75-1	brain	PC12, brain
Reactivity on WB	+	-	-	-

REFERENCE:

1) Messam, C. A., *et al.*, *Exp. Neurol*. **161**, 585-596 (2000)



Western blot analysis of Human Nestin expression in U251 cells (1), SK-N-SH cells (2), Jurkat cells (3), Raji cells (4), Mouse brain (5), Rat brain (6) and PC12 cells (7) using K0199-3.



Western blot analysis of Human Nestin expression in U87/MG cells (1), HeLa cells (2) and ZR75-1 cells (3) using K0199-3.

MBL MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
URL https://ruo.mbl.co.jp
e-mail support@mbl.co.jp, TEL 052-238-1904

PROTOCOLS:

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°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. Measure the protein concentration of the supernatant and add the Lysis buffer to make 5 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/cm^2 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 primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 6 times).
9) Incubate the membrane with the 1:5,000 HRP-conjugated anti-mouse IgG (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS-T (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 10 minutes. Develop the film as usual. The condition for exposure and development may vary.

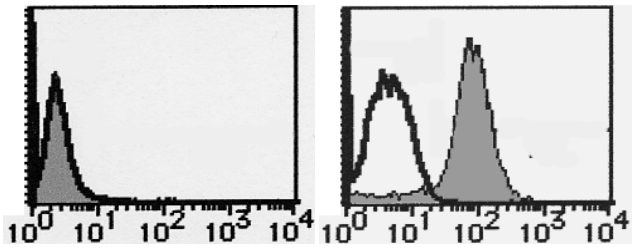
(Positive controls for Western blotting; U251, SK-N-SH, U87/MG)

Flow cytometric analysis for floating cells

We usually use Fisher tubes or equivalents as reaction tubes for all step 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 10 μL of normal goat serum containing 1 mg/mL normal human IgG and 0.1% NaN_3 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 of as suggest 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 30 μL of 1:40 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; SK-N-SH)



Flow cytometric analysis of Human Nestin expression in SK-N-SH cells (right) and Jurkat cells (left). *Open histograms indicate the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of K0199-3 to the cells.*

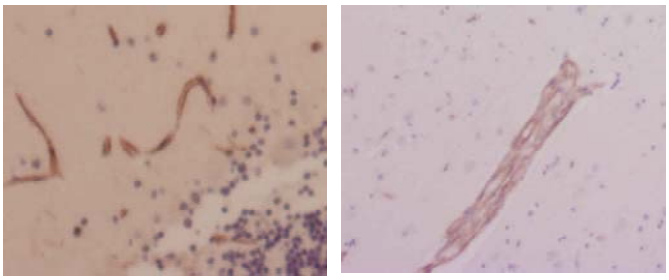
Immunohistochemical staining for paraffin embedded sections: SAB method

1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
2) Wash the slides with Ethanol 3 times for 3-5 minutes each.
3) Wash the slides with PBS 3 times for 3-5 minutes each.
4) Heat treatment
   Heat treatment by microwave oven:
   Place the slides put on staining basket in 500 mL beaker with 500 mL of 10 mM citrate buffer (pH 6.5). Cover the beaker with plastic wrap, then process the slides 2 times for 10 minutes each at 500 W with microwave oven. Let the slides cool down in the beaker at room temperature for about 40 minutes.
5) Remove the slides from the citrate buffer and cover each

section with 3% H_2O_2 for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 3 times in PBS for 5 minutes each.

6) Remove the slides from PBS, wipe gently around each section and cover tissues with Protein Blocking Agent (Ultratech HRP Kit; MBL, code no. IM-2391) for 5 minutes to block non-specific staining. Do not wash.
7) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with PBS containing 1% BSA as suggest in the **APPLICATIONS**.
8) Incubate the sections for 1 hour at room temperature.
9) Wash the slides 3 times in PBS for 5 minutes each.
10) Wipe gently around each section and cover tissues with Polyvalent Biotinylated Antibody (Ultratech HRP Kit). Incubate for 10 minutes at room temperature. Wash as in step 9).
11) Wipe gently around each section and cover tissues with Streptavidin-Peroxidase (Ultratech HRP Kit). Incubate for 10 minutes at room temperature. Wash as in step 9).
12) Visualize by reacting for 10-20 minutes with substrate solution containing 7.5 mg DAB, 40 μL of 30% H_2O_2 in 150 mL PBS. *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
13) Wash the slides in water for 5 minutes.
14) Counter stain in hematoxylin for 1 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes. Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each.
15) Now ready for mounting.

(Positive control for Immunohistochemistry; Cerebrum, Cerebellum)



Immunohistochemical detection of Human Nestin on paraffin embedded sections of Human normal cerebellum (Left) and cerebral cortex (Right) with K0199-3.

RELATED PRODUCTS:

D097-3	Anti-GFAP (MO389)
D097-3S	Anti-GFAP (MO389)
D098-3	Anti-Phosphorylated GFAP Thr7 (TMG7)
D098-3S	Anti-Phosphorylated GFAP Thr7 (TMG7)
MY-01-3	Anti-Phospho GFAP Ser8 (YC10)
D121-3	Anti-Phosphorylated GFAP Ser13 (KT13)
K0104-3	Anti-Desmoglein 2 (6D8)
D217-3	Anti-mouse Desmoglein 3 (AK9)
D218-3	Anti-mouse Desmoglein 3 (AK18)
D219-3	Anti-mouse Desmoglein 3 (AK23)