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

MONOCLONAL ANTIBODY

Anti-Musashi-1 (Msi1) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D270-3	14H1	Rat IgG2a κ	100 µL	1 mg/mL

BACKGROUND: Musashi-1 (Msi1) is a highly conserved RNA binding protein with important functions in stem cell maintenance, nervous system development and tumorigenesis. Musashi-1 inhibit translation initiation of its target mRNA, *m-Numb*, by competing with eIF4G for Poly A Binding Protein. Musashi-1 is mainly expressed in stem and progenitor cells from different tissues in mammals, and a decrease occurs as cells commit to lineage differentiation. Recently, expression of Musashi-1 in mature photoreceptors and retinal pigment epithelium cells was found and its expression in those cells is absolutely necessary for survival of photoreceptors.

SOURCE: This antibody was purified from hybridoma (clone 14H1) supernatant using protein G agarose. This hybridoma was established by fusion of mouse myeloma cell P3X63Ag8U.I with Wister rat splenocyte immunized with the recombinant protein containing 190-362 aa peptide of full length mouse Musashi-1. The immunized protein was lacked two alternative splicing regions (245-268 and 287-297). 14H1 epitope lies between the Musashi-1 amino acids 235 and 244 (LAPGYTYQFP).

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 Musashi-1 on Western blotting and Immunohistochemistry.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	SK-N-SH, IMR-32	P19*, Transfectant	PC12**
Reactivity on WB	+	+	+

*Retinoic acid treated
**Nerve Growth Factor treated

INTENDED USE:
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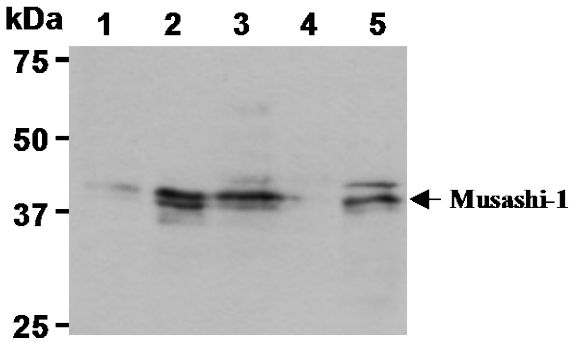
APPLICATIONS:
Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; Not recommended
Immunohistochemistry; 2 µg/mL
Heat treatment is necessary for paraffin embedded sections.
Microwave oven; 2 times for 10 minutes each in 10 mM citrate buffer (pH 6.3)
Immunocytochemistry; Not tested
*It is reported that this antibody can be used in this application in the reference number 1).
Flow cytometry; Not tested

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

REFERENCES:
1) Yousefi, M., *et al., J. Cell Biol.* **215**, 401-413 (2016) [IC, IHC]
2) Katz, Y., *et al., Elife*. **3**, e03915 (2014) [IHC]
3) Takeda, H., *et al., Genesis.* **51**, 128-134 (2013) [IHC]
4) Susaki, K., *et al., Exp. Eye Res.* **88**, 347-355 (2009)
5) Abreu, R. S., *et al., J. Biol. Chem.* **284**, 12125-12135 (2009)
6) Kawahara, H., *et al., J. Cell Biol.* **181**, 639-653 (2008)
7) Imai, T., *et al., Mol. Cell Biol*. **21**, 3888-3900 (2001)
8) Kaneko, Y., *et al., Dev. Neurosci*. **22**, 139-153 (2000)
9) Sakakibara, S., *et al., Dev. Biol*. **176**, 230-242 (1996)

Clone 14H1 was established by Kaneko *et al.* and reported in the reference number 8).



Western blot analysis of Musashi-1 expression on SK-N-SH (1), P19 (retinoic acid treated, 2), IMR-32 (3), U251 (4) and PC12 (NGF treated, 5) using D270-3.

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PROTOCOLS:

SDS-PAGE & Western Blotting

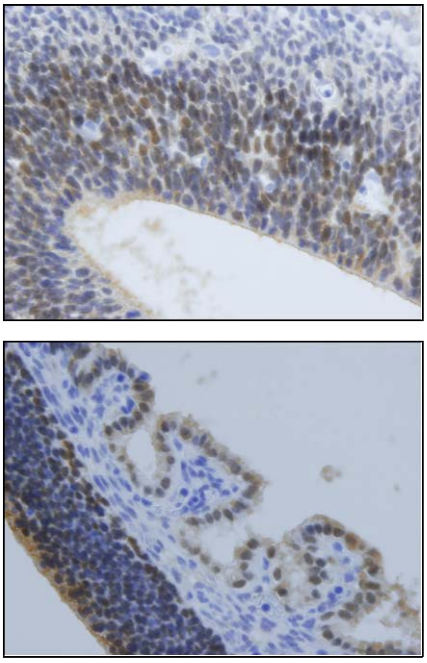
1) Wash 1 x 10^7 cells 3 times with PBS and resuspend them in 1 mL of Laemmli's sample buffer.
2) Boil the samples for 2 minutes and centrifuge. Load 20 µL of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
3) Blot the protein to a polyvinylidene difluoride (PVDF) 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 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
5) Incubate the membrane for 1 hour at room temperature with primary antibody diluted with PBS (pH 7.2) containing 1% skimmed milk as suggested in the **APPLICATIONS**. (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 HRP-conjugated anti-rat IgG antibody 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 off the membrane, and incubate membrane with an appropriate chemiluminescence reagent for 1 minute.
10) Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
11) Expose the membrane onto an X-ray film in a dark room for 10 minutes. Develop the film under usual settings. The conditions for exposure and development may vary.

(Positive controls for Western blotting; SK-N-SH, IMR-32, U251, P19 and PC12)

Immunohistochemical staining for paraffin-embedded sections

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:
   Place the slides put on staining basket in 500 mL beaker with 500 mL of 10 mM citrate buffer (pH 6.3). 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 in PBS 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 blocking buffer (20 mM HEPES, 1% BSA, 135 mM NaCl) 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 blocking buffer as suggested 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 ENVISION+Dual Link (DAKO; code no. K4063). Incubate for 1 hour at room temperature. Wash as in step 9).
11) Visualize by reacting for 10 minutes with DAB substrate solution (DAKO; code no. K3465). *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
12) Wash the slides in water for 5 minutes.
13) 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.
14) Now ready for mounting.

(Positive control for Immunohistochemistry; Mouse embryonic brain)



Immunohistochemical detection of mouse Musashi-1 on paraffin embedded section of mouse embryonic brain (E14.5) with D270-3.

RELATED PRODUCTS:

D270-6	Anti-Musashi-1 (Msi1) mAb-Biotin (14H1)
RN010P	Anti-MSI1 (Musashi1) pAb (polyclonal)
M081-3	Rat IgG2a (isotype control) (2H3)