

MONOCLONAL ANTIBODY

Anti-HES1 mAb

Code No.	Clone	Subclass	Quantity	Concentration
D134-3	NM1	Rat IgG2a	100 µL	1 mg/mL

BACKGROUND: Two transcription factors with a helix-loop-helix motif, human achaete-scute homologue-1 (hASH1) and hairy/enhancer of split homologue-1 (HES1), are essential for proper neuronal development. hASH1 is constitutively expressed in the most virulent human lung cancer, small cell lung cancer (SCLC), which exhibits neuroendocrine (NE) features. Moreover, hASH1 is essential for development of normal pulmonary NE cells that resemble SCLC. HES1 acts to transcriptionally repress hASH1 expression. The repressive effect of HES1 is mediated by binding of the protein to a class C site in the hASH1 promoter. HES1 also plays a role in mid- and hindbrain development, and in Notch signaling in thymocytes.

SOURCE: This antibody was purified from hybridoma (clone NM1) supernatant using protein G agarose. This hybridoma was established by fusion of mouse myeloma cell SP2/0 with Wister rat iliac lymphocyte immunized with the full-length rat HES1 (1-281 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 HES1 on Western blotting, Immunoprecipitation and Immunocytochemistry.

APPLICATIONS:

Western blotting; 0.1-1 µg/mL

Immunoprecipitation; 2 µg/300 µL of cell extract

Immunohistochemistry; Not tested*

*It is reported that clone NM1 can be used in this application in the reference number 2)-3) using human samples and 4), 6)-7) using mouse samples. Optimization will be required by end user. MBL has not validated this antibody for immunohistochemistry and does not guarantee or warrant reactivity.

Immunocytochemistry; 10-20 µg/mL

Flow cytometry; Not tested

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	Not tested*	NIH/3T3	Transfectant
Reactivity on WB		-	+

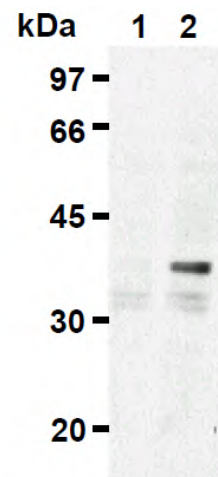
*It is reported that clone NM1 can be used in Western blotting in the reference number 5) using human sample. Optimization will be required by end user. MBL has not validated this antibody for other sample types and does not guarantee or warrant reactivity.

INTENDED USE:

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

REFERENCES:

- 1) Shitamukai, A., *et al. J. Neurosci.* **31**, 3683-3695 (2011) [IHC]
- 2) Meliou, E., *et al. J. Endod.* **37**, 176-180 (2011) [IHC]
- 3) Meliou, E., *et al. Open Dent. J.* **4**, 153-158 (2010) [IHC]
- 4) Cao, L., *et al., Biomaterials* **30**, 4085-4093 (2009) [IHC]
- 5) Rustighi, A., *et al., Nat. Cell Biol.* **11**, 133-142 (2009) [WB, IP]
- 6) Vasyutina, E., *et al., PNAS* **104**, 4443-4448 (2007) [IHC]
- 7) Ridgway, J., *et al., Nature* **444**, 1083-1087 (2006) [IHC]
- 8) Murata, K., *et al., Mol. Cell. Biol.* **25**, 4262-4271 (2005)
- 9) Tomita, K., *et al., Genes Dev.* **13**, 1203-1210 (1999)



Western blot analysis of HES1 expression in HES1 transfected HeLa cells(+Dox) (1) and HES1 transfected HeLa cells(-Dox) (2) using D134-3.

The descriptions of the following protocols are examples.
Each user should determine the appropriate condition.

PROTOCOLS:

SDS-PAGE & Western Blotting

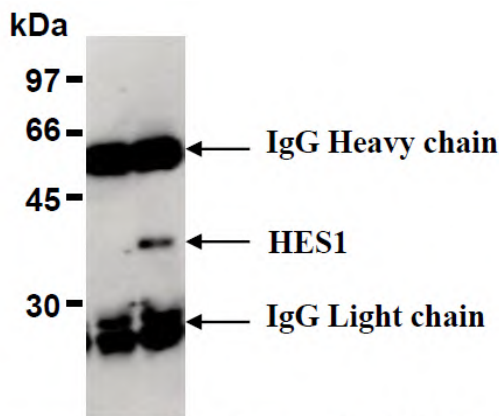
- 1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
- 2) Centrifuge 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 cold Lysis buffer to make 8 mg/mL solution.
- 3) Mix the sample with equal volume of Laemmli's sample buffer.
- 4) Boil the samples for 3 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/cm² 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 3 times).
- 9) Incubate the membrane with the 1:4,000 HRP-conjugated anti-rat IgG diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
- 10) Wash the membrane with PBS-T (10 minutes x 3 times).
- 11) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 minute.
- 12) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 13) Expose to an X-ray film in a dark room for 3 minutes.
- 14) Develop the film as usual. The condition for exposure and development may vary.

Immunoprecipitation

- 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.
- 3) Add primary antibody as suggest in the **APPLICATIONS** into 300 µL of the supernatant. Mix

well and incubate with gentle agitation for 30-120 minutes at 4°C.

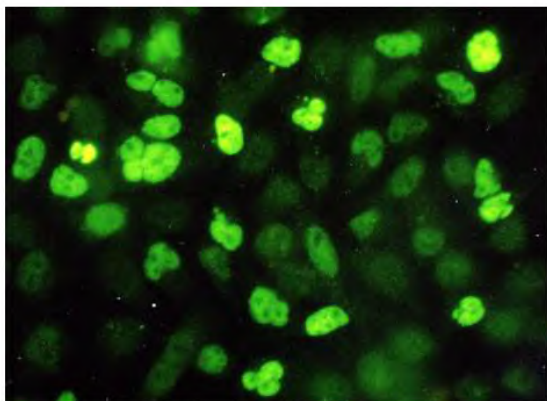
- 4) Add 20 µL of 50% protein G agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
- 5) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
- 6) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10 µL/lane for the SDS-PAGE analysis. (See **SDS-PAGE & Western blotting**.)



Immunoprecipitation of HES1 from HES1 transfected HeLa cells(+Dox) (1) and HES1 transfected HeLa cells(-Dox) (2) with D134-3. After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with D134-3.

Immunocytochemistry

- 1) Culture the cells in the appropriate condition on a glass slide. (For example, spread 1x10⁴ cells for one slide, and then incubate in a CO₂ 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 for 10 minutes on ice.
- 4) The glass slide was washed 3 times with PBS.
- 5) Immerse the slide in PBS containing 0.1% Triton X-100 for 10 minutes at room temperature.
- 6) The glass slide was washed 3 times with PBS.
- 7) Add the primary antibody diluted with PBS as suggest 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.)
- 8) The glass slide was washed 3 times with PBS.
- 9) Add 100 µL of 1:40 FITC conjugated anti-rat IgG diluted with PBS onto the cells. Incubate for 1 hour at room temperature. Keep out light by aluminum foil.
- 10) The glass slide was washed 3 times with PBS.
- 11) Wipe excess liquid from 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.



***Immunocytochemical detection of HES1
on HES1 transfected HeLa cells(-Dox)
using D134-3.***

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