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MONOCLONAL ANTIBODY

Anti-Np95 (Uhrf1) (Mouse) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D289-3	Th-10a	Rat IgG2a κ	100 µL	1 mg/mL

BACKGROUND: DNA methylation inheritance is the process of copying pre-existing methylation patterns onto newly replicated DNA strand after DNA replication. Dnmt1 is the maintenance DNA methyltransferase, which methylates hemimethylated CpGs that appear after DNA repairs and replications. The mouse Np95, also known as Uhrf1, is a nuclear protein, which contains an ubiquitin-like domain, a tandem Tudor domain, a PHD (Plant Homeodomain) finger, an SRA (SET-and RING-associated) domain, and a RING (Really Interesting New Gene) finger domain. Np95 recognizes hemimethylated CpGs via the SRA domain, and recruits Dnmt1 to the sites to transfer the methylation patterns onto the newly synthesized DNA strand. Therefore, the interaction between Np95 and Dnmt1 is necessary for the inheritance of DNA methylation.

SOURCE: This antibody was purified from a hybridoma (clone Th-10a) supernatant using protein G agarose. This hybridoma was established by fusion of mouse myeloma P3-X63-Ag8.653 cells with Wister Rat spleen cells immunized with a murine thymic lymphoma (TIGN) developed from B10.Thy1.1 mice 6 months after split-dose irradiation.

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 endogenous mouse Np95 (95 kDa).

APPLICATIONS:

Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; Not tested*
Flow cytometry; Not tested*
Immunocytochemistry; 2 µg/mL for fluorescence detection system
Immunohistochemistry (frozen section); Not tested*
*It is reported that Th-10a can be used in Immunoprecipitation, Immunohistochemistry and Flow cytometry in the reference number 8).
Detailed procedure is provided in the following **PROTOCOL**.

SPECIES CROSS REACTIVITY on WB or IC:

Species	Human	Mouse	Rat	Hamster
Cells		E14 ES cells (*Np95*$^{+/+}$), NIH/3T3		
Reactivity	-	+	-	-

Reactivity of Th-10a to other species is not confirmed in our laboratory. However, it is reported that this clone did not cross-react with human, rat and hamster Np95 in the reference number 8).

Entrez Gene ID: 18140 (Mouse)

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

REFERENCES:

1) Ohno, R., *et al., Development* **140**, 2892-2903 (2013)
2) Sharif, J., *et al., Nature* **450**, 908-912 (2007) [IC]
3) Muto, M., *et al., Radiat. Res*. **166**, 723-733 (2006)
4) Muto, M., *et al., J. Biol. Chem.* **277**, 34549-34555 (2002) [WB]
5) Bonapace, I. M., *et al., J. Cell Biol.* **157**, 909-914 (2002) [WB, IC]
6) Uemura, T., *et al., Cell Struct. Funct.* **25**, 149-159 (2000) [IC]
7) Fujimori, A., *et al., Mamm. Genome* **9**, 1032-1035 (1998) [WB]
8) Muto, M., *et al., Cell Prolif.* **28**, 645-657 (1995) [IP, IHC-f, FCM]

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

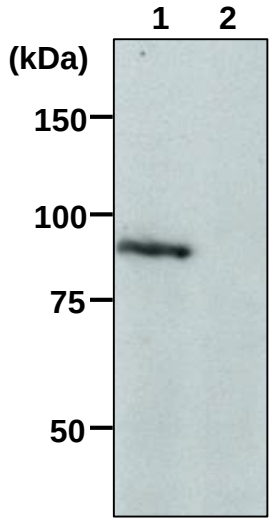
PROTOCOL:

SDS-PAGE & Western Blotting

1) Wash cells (approximately 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 min. and centrifuge. Load 10 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 hr. 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 hr. at room temperature, or overnight at 4°C.
5) Incubate the membrane for 1 hr. 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 min. x 3 times).
7) Incubate the membrane with 1:10,000 of anti-IgG (rat) pAb-HRP (MBL; code no. IM-0825) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
8) Wash the membrane with PBS-T (5 min. x 3 times).
9) Wipe excess buffer off the membrane, and incubate membrane with an appropriate chemiluminescence reagent for 1 min.
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 3 min. Develop the film under usual settings. The conditions for exposure and development may vary.

(Positive control for Western blotting; E14 ES cells)

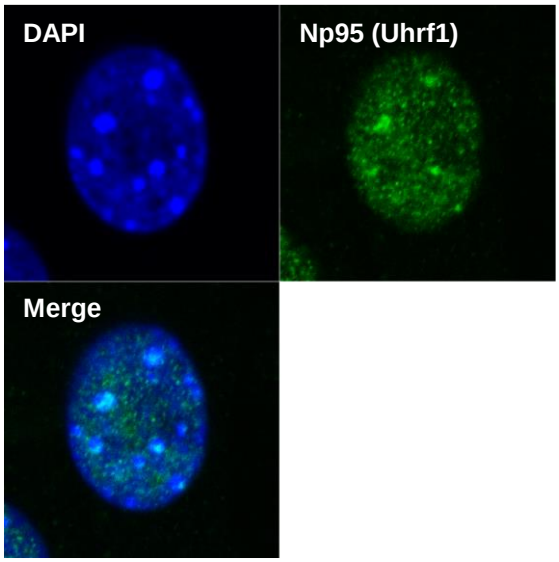


Western blot analysis for detecting Np95 expression in mouse ES cells E14 (Np95$^{+/+}$) (lane 1) and 19.4 (Np95$^{-/-}$) (lane 2) using D289-3.

Immunocytochemistry

1) Spread the cells on a glass slide, then incubate in a CO_2 incubator for one night.
2) Remove the culture supernatant by careful aspiration. Do not allow the slide to dry during step 2)-13).
3) Wash the slide with PBS (1 min. x 3 times).
4) Fix the cells with 4% paraformaldehyde (PFA)/PBS for 30 min. at room temperature (20~25°C).
5) Wash the slide with PBS (1 min. x 3 times).
6) Permeabilize the cells with 0.5% Triton X-100/PBS for 15 min. at room temperature.
7) Wash the slide with PBS (1 min. x 2 times).
8) Block the cells with 1x Block Ace (Snow Brand Milk Products) for 1 hr. at room temperature.
9) Wash the slide with PBS (1 min. x 1 time).
10) Tip off PBS and incubate the cells with the primary antibody diluted with PBS as suggested in the **APPLICATIONS** for overnight at 4°C. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
11) Wash the slide in PBS with gentle shaking for 1 hr. During this step, PBS should be changed more than 3 times.
12) Incubate the cells with 1:500 Alexa Fluor® 488 Goat Anti-rat IgG (Invitrogen; code no. A11006) diluted with PBS for 1 hr. at room temperature in dark chamber.
13) Wash the slide in the same way as step 11). Keep out light by aluminum foil.
14) Wipe excess liquid from the slide. (Take care not to touch the cells.)
15) Promptly add VECTASHIELD Mounting Medium with DAPI (Vector Laboratories; code no. H-1200) onto the slide, then put a cover slip on it.
16) Observe the slide using confocal laser scanning microscopy (Carl Zeiss; LSM510).

(Positive control for Immunocytochemistry; NIH/3T3)



Immunocytochemical detection of Np95 (Uhrf1) in NIH/3T3
Green: Anti-Np95 (Uhrf1) mAb (D289-3)
Cyan: DAPI
The data were kindly provided by Dr. Motoko Unoki Ph.D. (Division of Epigenomics and Development, Department of Molecular Genetics, Medical Institute of Bioregulation, Kyushu University)

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