

Anti-Np95 (Uhrf1) mAb

CODE No.	M202-3
CLONALITY	Monoclonal
CLONE	R32-44
ISOTYPE	Rat IgG2c κ
QUANTITY	100 μ L, 1 mg/mL
SOURCE	Purified IgG from hybridoma supernatant
IMMUNOGEN	Recombinant protein, corresponding to amino acids 590-713 of mouse Np95 (Uhrf1)
FORMURATION	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.

APPLICATIONS-CONFIRMED

<u>Western blotting</u>	1 μ g/mL for chemiluminescence detection system
<u>Immunocytochemistry</u>	3 μ g/mL

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Cell	HEK293T	E14 ES cells	Not tested	Not tested
Reactivity	+	+		

Entrez Gene ID 29128 (Human), 18140 (Mouse)

- REFERENCES**
- 1) Hashimoto, H., *et al.*, *Nature* **455**, 826-829 (2008)
 - 2) Arita, K., *et al.*, *Nature* **455**, 818-821 (2008)
 - 3) Bostick, M., *et al.*, *Science* **317**, 1760-1764 (2007)
 - 4) Sharif, J., *et al.*, *Nature* **450**, 908-912 (2007)
 - 5) Unoki, M., *et al.*, *Oncogene* **23**, 7601-7610 (2004)

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RELATED PRODUCTS

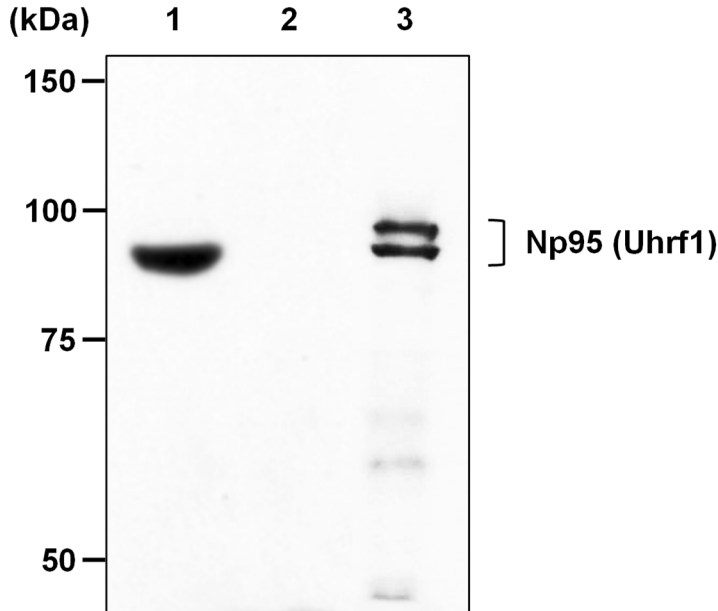
M202-3	Anti-Np95 (Uhrf1) mAb (R32-44)
D289-3	Anti-Np95 (Uhrf1) (Mouse) mAb (Th-10a)
MH-12-3	Anti-PCNA mAb (5A10)
MK-15-3	Anti-Rb (Human) mAb (3H9)
D141-3	Anti-G9a mAb (14-1)
D209-3	Anti-Histone H1 mAb (C14093)
D210-3	Anti-Histone H2A mAb (C10037)
D212-3	Anti-Histone H2B mAb (C14264)
D214-3	Anti-Histone H4 mAb (C14691)
PM006	Anti-Phospho-Histone H3 (Ser28) (Human) pAb
CY-P1015	Anti-Phospho-Histone-H2A.X (Ser139) pAb
CY-P1011	Anti-HDAC1 (Histone Deacetylase 1) pAb
CY-P1012	Anti-HDAC2 (Histone Deacetylase 2) pAb
M041-3	Anti-PML (Human) mAb (1B9)
K0196-3	Anti-PML (Mouse) mAb (36-1-104)
PM001	Anti-PML (Human) pAb
5270-100	MethylHunter MBD1-based Methylated DNA Enrichment Kit
5275-100	MethylHunter MBD1-based Methylated DNA Enrichment Kit 2

Other related antibodies and kits are also available.
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SDS-PAGE & Western blotting

- 1) Wash 5×10^6 cells 3 times with PBS and resuspend them in 250 μ L of Extraction buffer (50 mM Tris-HCl pH 7.4, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA), then sonicate for 30 sec.
- 2) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant.
- 3) Add equal volume of 2 x Laemmli's sample buffer and mix well.
- 4) Boil for 5 min., centrifuge, and collect supernatant.
- 5) Load the sample in a 1-mm-thick SDS-polyacrylamide gel (7.5% acrylamide) for electrophoresis.
- 6) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm² 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.
- 7) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
- 8) Incubate the membrane with primary antibody diluted with PBS (pH 7.2) containing 1% skimmed milk as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
- 9) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
- 10) Incubate the membrane with 1:10,000 of anti-IgG (rat) pAb-HRP (Millipore; code no. AP136P) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
- 11) Wash the membrane with PBS-T (5 min. x 3 times)
- 12) Wipe excess buffer on the membrane, then incubate it with ECLTM WesternBlotting Detection Reagents (GE Healthcare; code no. RPN2106) for 1 min. 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 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; Mouse E14 ES cells and HEK293T)



Western blot analysis of Np95 (Uhrf1)

- Lane 1: E14 ES cells (20 μ g of whole cell lysate)
- Lane 2: E14 *Np95*^{-/-} ES cells (20 μ g of whole cell lysate)
- Lane 3: HEK293T (40 μ g of whole cell lysate)

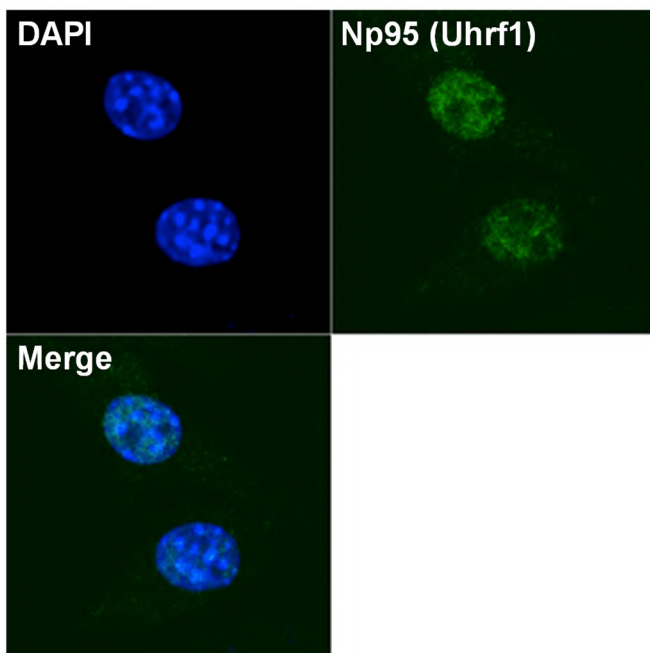
Immunoblotted with Anti-Np95 (Uhrf1) mAb (M202-3)

E14 ES cells were provided by Dr. Haruhiko Koseki, M.D. Ph.D.
(Developmental Genetics Laboratories, RIKEN)

Immunocytochemistry

- 1) Spread the cells on a glass slide, then incubate in a CO₂ 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)

Green: Np95 (Uhrf1)
Cyan: DAPI

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)