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

Anti-Np95 (Uhrf1) mAb

CODE No. M202-3

CLONALITY Monoclonal
CLONE R32-44
ISOTYPE Rat IgG2c κ
QUANTITY 100 µL, 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

Western blotting	1 µg/mL for chemiluminescence detection system
Immunocytochemistry	3 µg/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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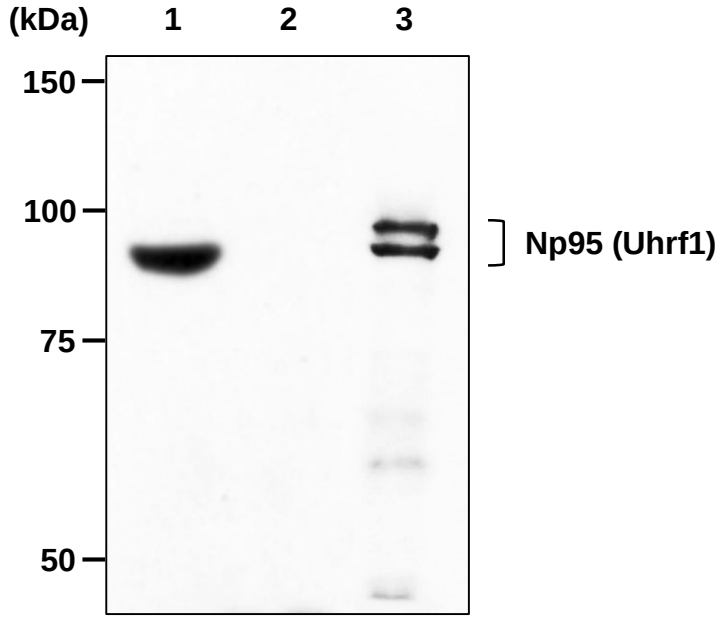
SDS-PAGE & Western blotting

1) Wash 5 x 10^6 cells 3 times with PBS and resuspend them in 250 μL 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/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.
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 ECL™ 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 μg of whole cell lysate)
Lane 2: E14 *Np95*$^{-/-}$ ES cells (20 μg of whole cell lysate)
Lane 3: HEK293T (40 μg of whole cell lysate)

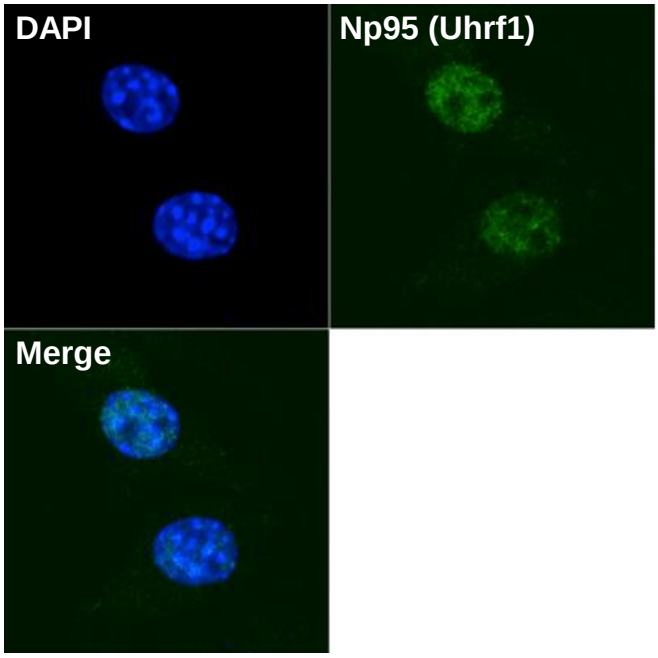
Immunoblotted with Anti-Np95 (Uhrf1) mAb (M202-3)

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 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)

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)