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

MONOCLONAL ANTIBODY

Anti-Nuf2 (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
M146-3	4H9	Mouse IgG2a	100 µL	1 mg/mL

BACKGROUND: Nuf2, a widely conserved protein in eukaryote, compose the Ndc80 complex with NDC80/HEC1, SPC24 and SPC25. The Ndc80 complex is a constituent of the outer plate of kinetochore and acts to establish kinetochore-spindle connection at centromere for chromosome segregation in mitosis. Nuf2 localizes to kinetochores from late prophase to anaphase.

SOURCE: This antibody was purified from hybridoma (clone 4H9) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with C3H mouse lymphocyte immunized with the recombinant full-length human Nuf2 (1-464 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 Nuf2 (54 kDa) on Western blotting and Immunoprecipitation.

APPLICATIONS:

Western blotting; 10 µg/mL for chemiluminescence detection system
Immunoprecipitation; Not recommended
Immunohistochemistry; Not tested
Immunocytochemistry; Not recommended
Flow cytometry; Not tested

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	293T	Not tested	Not tested
Reactivity on WB	+		

INTENDED USE:

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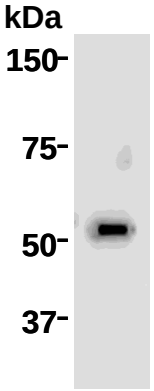
REFERENCES:

1) Ngo, B., *et al., J. Biol. Chem.* **288**, 34403-34413 (2013) [WB]
2) Wu, G., *et al., Mol. Cell Biol.* **20**, 4686-4695 (2009) [WB, IP]
3) Ciferri, C., *et al., J. Biol. Chem*. **280**, 29088-29095 (2005)
4) Wigge, P. A., *et al., J. Cell Biol*. **152**, 349-360 (2001)
5) Janke, C., *et al., EMBO J*. **20**, 777-791 (2001)
6) Osborne, M. A., *et al., J. Cell Biol*. **125**, 853-866 (1994)



Western blot analysis of Nuf2 expression in 293T using M146-3.

PROTOCOLS:

SDS-PAGE & Western Blotting

1) Wash the $5x10^6$ cells 3 times with PBS and suspend with 500 µL of Laemmli's sample buffer.
2) Boil the samples for 2 minutes and centrifuge. Load 5 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for 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 manufacture'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 hour at room temperature, or overnight at 4°C.
5) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% BSA, 0.1% Tween-20 as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
7) Incubate the membrane with 1:5,000 of Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% BSA (in

PBS, pH 7.2) for 1 hour at room temperature.
8) Wash the membrane with PBS-T (10 minutes x 3 times).
9) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 2 minute.
10) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
11) Expose to an X-ray film in a dark room for 3 minutes.
12) Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; 293T)

RELATED PRODUCTS:

D115-3	Anti-CENP-A (Human) mAb (3-19)
PD030	Anti-CENP-C (Human) pAb (polyclonal)
K0171-3	Anti-CENP-E (Human) mAb (1H12)
PD031	Anti-CENP-H (Human) pAb (polyclonal)
PD032	Anti-CENP-I (hMis6) (Human) pAb (polyclonal)
D282-3	Anti-CENP-K (ICEN37) (Human) mAb (46F1)
PD018	Anti-CENP-K (ICEN37) (Human) pAb (polyclonal)
D283-3	Anti-CENP-L (ICEN33) (Human) mAb (27E10)
D284-3	Anti-CENP-M (ICEN39) (Human) mAb (23F6)
D285-3	Anti-CENP-N (ICEN32) (Human) mAb (22F4)
D286-3	Anti-CENP-T (ICEN22) (Human) mAb (42F10)
PD019	Anti-CENP-50 (Human) pAb (polyclonal)
PD020	Anti-CENP-O (Chicken) pAb (polyclonal)
M076-3	Mouse IgG2a (isotype control) (6H3)