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

Anti-NFIL3 (E4BP4) pAb

CODE No. PM097

CLONALITY Polyclonal
ISOTYPE Rabbit Ig, affinity purified
QUANTITY 100 µL

SOURCE Purified Ig from rabbit serum
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.

APPLICATION-CONFIRMED

Western blotting 1:1,000 for chemiluminescence detection system

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Samples	HepG2, U2OS	Liver nuclear extract, NIH/3T3	Rat-1	Not tested
Reactivity	+	+	-	

Entrez Gene ID 4783 (Human), 18030 (Mouse)

REFERENCES

1) Yu, X., *et al.*, *Science* **342**, 727-730 (2013)
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3) Kashiwada, M., *et al.*, *PNAS*. **107**, 821-826 (2010)
4) Mitsui, S., *et al.*, *Genes Dev*. **15**, 995-1006 (2001)
5) Ikushima, S., *et al.*, *PNAS.* **94**, 2609-2614 (1997)
6) Zhang, W., *et al.*, *Mol. Cell Biol.* **15**, 6055-6063 (1995)
7) Cowell, I. G., *et al.*, *Mol. Cell Biol.* **12**, 3070-3077 (1992)

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MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
URL http://ruo.mbl.co.jp/
e-mail support@mbl.co.jp, TEL 052-238-1904

RELATED PRODUCTS

Antibodies

M225-3	Anti-NFIL3 (E4BP4) chimeric mAb (42)
PM097	Anti-NFIL3 (E4BP4) pAb
M219-3	Anti-ROR³t mAb (4H11)
PM080	Anti-ROR³t pAb
D333-3	Anti-CLOCK (Mouse) mAb (CLSP3)
D334-3	Anti-CLOCK (Mouse) mAb (CLNT1)
D349-3	Anti-CLOCK (Mouse) mAb (CLSP4)
D335-3	Anti-BMAL1 (Mouse) mAb (B1BH2)
PM091	Anti-Per1 (Mouse) pAb
PM083	Anti-Per2 (Mouse) pAb
PM096	Anti-PER2 (Human) pAb
PM082	Anti-Cry2 (Mouse) pAb
PM081	Anti-Cry1 (Mouse) pAb
PM079	Anti-DBP (Mouse) pAb
PM093	Anti-NR1D2 (Rev-erb²) pAb
PM092	Anti-NR1D1 (Rev-erb±) pAb
CY-P1016	Anti-SIRT1 pAb
RN032P	Anti-CIRBP pAb
RN013MW	Anti-Nono (P54NRB) mAb (C5)
RN092PW	Anti-NONO (P54NRB) pAb
RN014MW	Anti-SFPQ (PSF) mAb (C23)
RN106PW	Anti-SFPQ (PSF) pAb
RN015MW	Anti-PSPC1 (PSP1) mAb (1L4)
PM075	Anti-GNAT2 (Zebrafish) pAb

Kits

CY-1151	CycLex® SIRT1/Sir2 Deacetylase Fluorometric Assay Kit
CY-1152	CycLex® SIRT2 Deacetylase Fluorometric Assay Kit
CY-1173	CycLex® CaM-kinase II Assay Kit
CY-8102	CircuLex Mouse CIRP ELISA Kit
CY-8103	CircuLex Human CIRP ELISA Kit

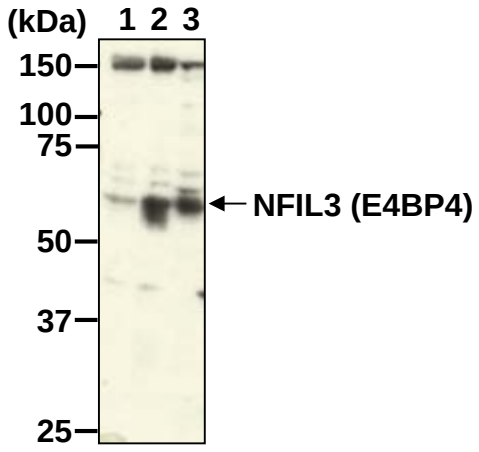
Recombinant proteins (Human, Active)

CY-E1151	NAD^+-Dependent Deacetylase SIRT1
CY-E1152	NAD^+-Dependent Deacetylase SIRT2
CY-E1173	CaM-kinase II Positive Control

SDS-PAGE & Western blotting

1) Prepare the samples described as below:
   [Tissue] Mix 10 μL of mouse liver nuclear extract with 10 μL of Laemmli's sample buffer.
   [Cell line] Wash 1 x 10^7 cells 3 times with PBS and suspends them in 1 mL of Laemmli's sample buffer.
2) Boil the sample for 5 min. and centrifuge. Load 10 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (10% acrylamide) for electrophoresis.
3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 190 mA 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 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
5) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
6) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATION** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
7) Wash the membrane with PBS-T (5 min. x 3 times).
8) Incubate the membrane with 1:10,000 of Anti-IgG (Rabbit) pAb-HRP (MBL; code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
9) Wash the membrane with PBS-T (5 min. x 3 times).
10) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min. 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 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; Mouse liver nuclear extract and HepG2)



Western blot analysis of NFIL3 (E4BP4)
Lane 1: Mouse liver nuclear extract, ZT12 (zeitgeber time; 12 h)
Lane 2: Mouse liver nuclear extract, ZT24 (zeitgeber time; 24 h)
Lane 3: HepG2

Immunoblotted with Anti-NFIL3 (E4BP4) pAb (PM097)