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

Anti-NFIL3 (E4BP4) chimeric mAb

CODE No. M225-3

CLONALITY Monoclonal
CLONE 42
ISOTYPE Guinea pig Ig κ, Rabbit Fc (chimeric)*
*Please use anti-rabbit IgG antibody as a secondary antibody.
QUANTITY 100 µL, 1 mg/mL

SOURCE Purified IgG from transfectant
IMMUNOGEN Recombinant protein, corresponding to internal region of mouse Nfil3 (E4bp4)
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	1-2 µg/mL for chemiluminescence detection system
Immunoprecipitation	1-2 µg/sample
Immunohistochemistry	Can be used.
Flow cytometry	1 µg/mL

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Sample	HepG2	Liver nuclear extract	Rat1	Not tested
Reactivity	+	+	–	

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

REFERENCES

1) Yu, X., *et al.*, *Science* **342**, 727-730 (2013)
2) Firth, M. A., *et al.*, *J. Exp. Med.* **210**, 2981-2990 (2013)
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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RELATED PRODUCTS

Antibodies

M225-3	Anti-NFIL3 (E4BP4) chimeric mAb (42)
M219-3	Anti-RORγt mAb (4H11)
PM080	Anti-RORγt pAb
D333-3	Anti-CLOCK (Mouse) mAb (CLSP3)
D334-3	Anti-CLOCK (Mouse) mAb (CLNT1)
D335-3	Anti-BMAL1 (Mouse) mAb (B1BH2)
D349-3	Anti-CLOCK (Mouse) mAb (CLSP4)
PM079	Anti-DBP (Mouse) pAb
CY-P1016	Anti-SIRT1 pAb
RN032P	Anti-CIRBP pAb
PM075	Anti-GNAT2 (Zebrafish) pAb
PM081	Anti-Cry1 (Mouse) pAb
PM082	Anti-Cry2 (Mouse) pAb
PM083	Anti-Per2 (Mouse) pAb
PM087	Anti-Chrono (Mouse) pAb
PM091	Anti-Per1 (Mouse) pAb
PM092	Anti-NR1D1 pAb
PM093	Anti-NR1D2 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

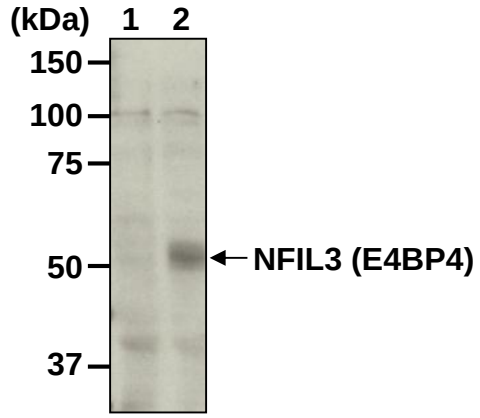
Recombinant proteins (Human, Active)

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.
1) Boil the samples for 5 min. and centrifuge. Load 20 μL (20 μg) of the tissue sample or 10 μL of the cell sample per lane in a 1-mm-thick SDS-polyacrylamide gel (10% acrylamide) for electrophoresis.
2) 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.
3) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS) overnight at 4°C.
4) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (3 times for 5 min.).
5) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
6) Wash the membrane with PBS-T (3 times for 5 min.).
7) Incubate the membrane with the 1:10,000 of Anti-IgG (Rabbit) pAb-HRP (MBL; code no. 458) diluted with 1% skimmed milk (in PBS) for 1 hr. at room temperature.
8) Wash the membrane with PBS-T (3 times for 5 min.).
9) Wash the membrane 1 time for 2 min. with PBS.
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. Develop the film as usual. The condition for exposure and development may vary.

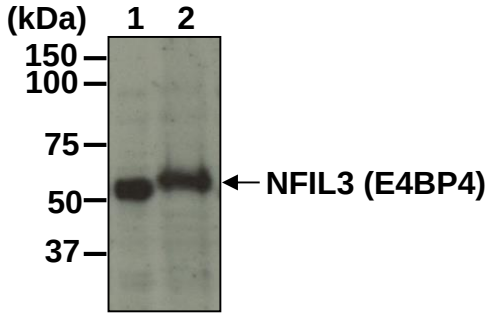
(Positive controls for Western blotting; HepG2 and mouse liver nuclear extracts)



Western blot analysis of mouse NFIL3 (E4BP4) from liver nuclear extract, ZT2 (zeitgeber time; 2 h)
Lane 1: *Nfil3* knockout
Lane 2: Wildtype
Exposure time: 15 min.

Immunoblotted with Anti-NFIL3 (E4BP4) mAb (M225-3)

Samples were kindly provided by Dr. Masaki Kashiwada. (Jichi Medical University Graduate School of Medicine)



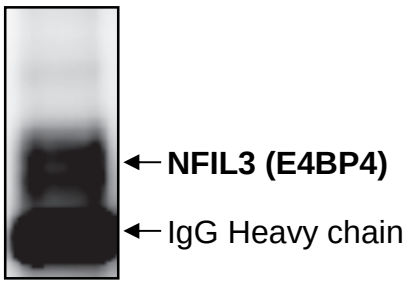
Western blot analysis of NFIL3 (E4BP4)
Lane 1: Mouse liver nuclear extract, ZT24 (zeitgeber time; 24 h)
Lane 2: HepG2
Exposure time: 3 min.

Immunoblotted with Anti-NFIL3 (E4BP4) mAb (M225-3)

Immunoprecipitation for tissue sample

1) Mix 100 μL of mouse liver nuclear extract to 200 μL of D1 buffer [20 mM HEPES-NaOH (pH 7.8), 5.5 mM NaCl, 1 mM EDTA, 6.5% glycerol, 1.5% Triton X-100, 1 mM DTT, 50 mM NaF, 1 mM Na_3VO_4] containing appropriate protease inhibitors.
2) Add 40 μL of 50% protein G agarose beads slurry resuspended in PBS. Incubate it at 4°C with rotation for 30 min.
3) Centrifuge the tube and transfer the supernatant to another tube.
4) Add primary antibody as suggested in the **APPLICATIONS** to 300 μL of precleared sample (prepared sample from step 3)). Incubate with gentle agitation for 1 hr. at 4°C.
5) Add 40 μL of 50% protein G agarose beads slurry in IP buffer [20 mM HEPES-NaOH (pH7.8), 140 mM NaCl, 1 mM EDTA, 5 % glycerol, 1.0 % TritonX-100, 1 mM DTT] containing appropriate protease inhibitors into the tube. Incubate with gentle agitation for 30 min. at 4°C.
6) Wash 1 time with 1 mL of IP buffer.
7) Resuspend the bead pellet in 8 μL of Laemmli's sample buffer, boil for 5 min. and centrifuge.
8) Load 8 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (7.4% acrylamide) for electrophoresis.
9) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 300 mA for 1 hr. using a Tanc Blotter. See the manufacturer's manual for precise transfer procedure.
10) To reduce nonspecific binding, soak the membrane in 1% Skimmed milk (in TBS) for 1 hr. at 37°C.
11) Incubate the membrane with 1 μg/mL of rabbit anti-mouse Nfil3 polyclonal antibody diluted with 1% Skimmed milk (in TBS) overnight at 4°C. (The concentration of antibody will depend on the conditions.)
12) Wash the membrane 3 times for 2 min., 5 min., 10 min. each with 1% Skimmed milk (in TBS).
13) Incubate the membrane with 1:5,000 of HRP-conjugated Donkey Anti-rabbit IgG (Kappel) diluted with 5% skimmed milk (in TBS) for 1 hr. at 37°C.
14) Wash the membrane 3 times for 2 min., 5 min., 10 min. each with TBS-T (TBS containing 0.05% Tween-20).
15) Wipe excess buffer on the membrane, and then incubate it with Western Lightning-Plus-ECL (Perkinelmer; code no. NEL105001EA) for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
16) Expose for 1-5 min. with ImageQuant LAS 4000 mini imaging system (Fujifilm). The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; Mouse liver nuclear extract)



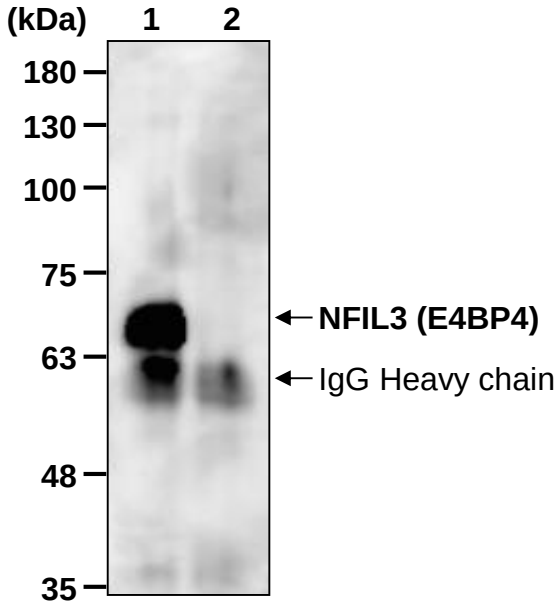
Immunoprecipitation of mouse NFIL3 (E4BP4) from liver nuclear extract

Data were kindly provided by Dr. Hikari Yoshitane and Dr. Yoshitaka Fukada.
(Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo)

Immunoprecipitation for cell samples

1) Wash cells 3 times with PBS and resuspend them with 800 μL of IP buffer [50 mM Tris (pH8.0), 150 mM NaCl, 1% NP-40] containing appropriate protease inhibitors. Mix well and incubate on ice for 20 min.
2) Centrifuge the tube at 12,000 xg for 10 min at 4°C. and transfer the supernatant to another tube.
3) Add 30 μL of 50% protein G agarose beads slurry resuspended in PBS. Incubate it at 4°C with rotation for 1 hr.
4) Centrifuge the tube and transfer the supernatant to another tube.
5) Add primary antibody as suggested in the **APPLICATIONS** to the supernatant. Incubate with gentle agitation for 3 hr. at 4°C.
6) Add 30 μL of 50% protein G agarose beads slurry into the tube. Incubate with gentle agitation for 1 hr. at 4°C.
7) Wash 4 times with 1 mL of IP buffer.
8) Resuspend the bead pellet in 30 μL of Laemmli's sample buffer, boil for 5 min. and centrifuge.
9) Load 30 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (7% acrylamide) for electrophoresis.
10) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 19 V for 45 min. using Trans-Blot SD Semi-Dry Electrophoretic Transfer Cell (Bio-Rad). See the manufacturer's manual for precise transfer procedure.
11) To reduce nonspecific binding, soak the membrane in blocking buffer [TBS containing 5% skimmed milk and 0.05% Tween-20) for 1 hr. at room temperature.
12) Wash the membrane 1 time for 5 min. with TBS-T (0.05% Tween-20 in TBS).
13) Incubate the membrane with 0.5 μg/mL of E4BP4 Antibody (Santa Cruz; code. no. sc-9550) diluted with blocking buffer overnight at 4°C. (The concentration of antibody will depend on the conditions.)
14) Wash the membrane 5 times for 5 min. each with TBS-T.
15) Incubate the membrane with 1:2,000 of HRP-conjugated Donkey Anti-Goat IgG (Santa Cruz; code. no. sc-2020) diluted with 5% skimmed milk (in TBS-T) for 1 hr. at room temperature.
16) Wash the membrane 5 times for 5 min. each with TBS-T.
17) Wipe excess buffer on the membrane, and then incubate it with WesternBright Sirius HRP substrate (Advansta) for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
18) Expose for 10-60 sec. with ImageQuant LAS 4000 imaging system (Fujifilm). The condition for exposure and development may vary.

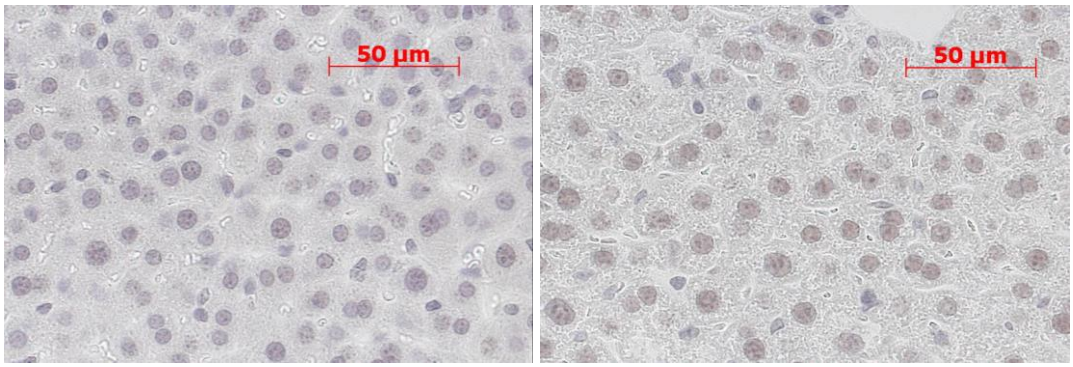
(Positive control for Immunoprecipitation; Wildtype D10.G4.1)



Immunoprecipitation of mouse NFIL3 (E4BP4)
Lane 1: Wildtype D10.G4.1 cells
Lane 2: *Nfil3* knockout D10.G4.1 cells

Data were kindly provided by Dr. Masaki Kashiwada.
(Jichi Medical University Graduate School of Medicine)

Immunohistochemical staining for paraffin embedded section



Immunohistochemical detection of NFIL3 (E4BP4) in mouse liver

Brown: Anti-NFIL3 (E4BP4) mAb (M225-3), 5 µg/mL
Blue: Hematoxylin

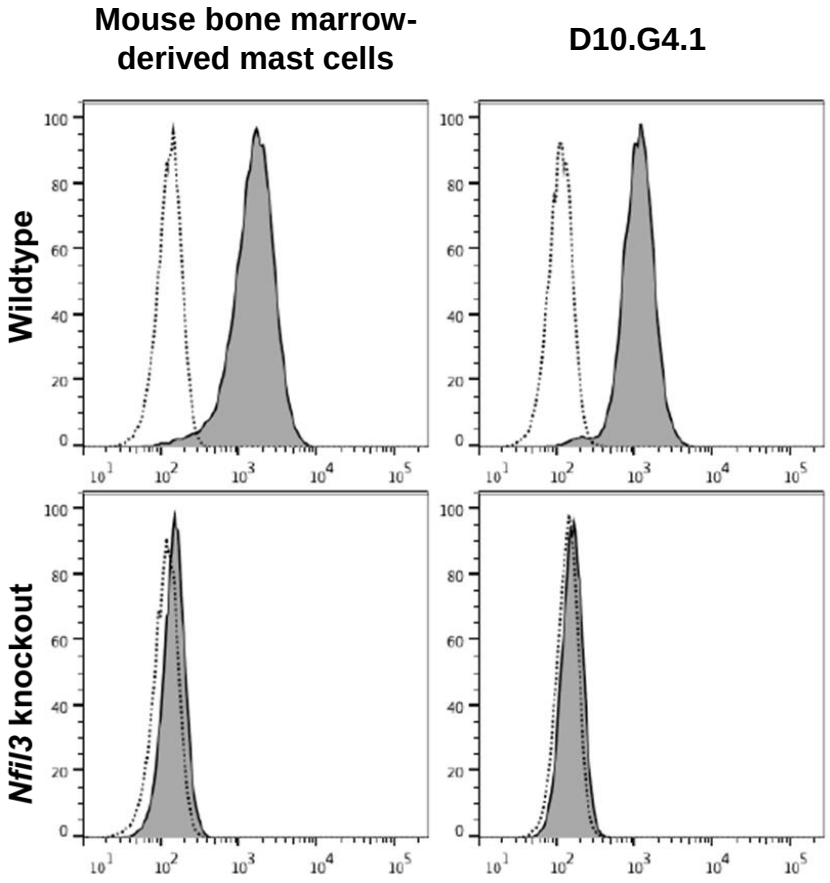
Left: ZT12 (zeitgeber time; 12 h)
Right: ZT24 (zeitgeber time; 24 h)

Antigen retrieval: Heat-treated (1 mM EDTA (pH8.0) , 121℃, 5 min.)

Flow cytometric analysis

1) Wash the cells (5 x 10^5 cells/sample) 3 times with 200 µL of PBS containing 0.5% fetal calf serum (FCS).
2) Prepare Fixation/Permeabilization Buffer by diluting Fixation/Permeabilization Concentrate (eBioscience; code no. 00-5123) with Fixation/Permeabilization Diluent (eBioscience; code no. 00-5223).
3) Add 200 µL of Fixation/Permeabilization Buffer to the cell pellet. Mix well and incubate on ice for 30 min.
4) Wash the cells 3 times with 200 µL of Permeabilization Buffer (eBioscience; code no. 00-8333).
5) Add 200 µL of 2% normal rat serum diluted with Permeabilization Buffer to the cell pellet. Mix well and incubate on ice for 15 min.
6) Add 100 µL of the primary antibody at the concentration as suggested in the **APPLICATION** diluted with Permeabilization Buffer. Mix well and incubate on ice for 30 min.
7) Wash the cells 3 times with 200 µL of Permeabilization Buffer.
8) Add 100 µL of 1 µg/mL Alexa Fluor® 488 Donkey anti-rabbit IgG (minimal x-reactivity) Antibody (Biolegend; code no. 406416) diluted with Permeabilization Buffer. Mix well and incubate on ice for 30 min.
9) Wash the cells 3 times with 350 µL of PBS containing 0.5% FCS.
10) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

(Positive control for flow cytometry; Wildtype mouse bone marrow-derived mast cell and D10.G4.1)



Flow cytometric analysis of NFIL3 (E4BP4)

Left: Mouse bone marrow-derived mast cell
Right: D10.G4.1

Open: Negative control (No primary antibody)
Closed: Anti-NFIL3 (E4BP4) mAb (M225-3)

Data were kindly provided by Dr. Masaki Kashiwada.
(Jichi Medical University Graduate School of Medicine)