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

My select sampler set

Anti-NRF2 pAb

CODE No. PM069MS

CLONALITY Polyclonal
ISOTYPE Rabbit Ig, affinity purified
QUANTITY 20 µL

SOURCE Purified Ig from rabbit serum
IMMUNOGEN Human NRF2, full length (recombinant)
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 1:1,000 for chemiluminescence detection system
Immunoprecipitation 5 µL/300 µL of cell extract from 3 x 10^6 cells
Immunocytochemistry 1:1,000
Immunohistochemistry 1:1,000 (paraffin section)
Heat treatment for paraffin embedded section: microwave oven, for 20 min. in 10 mM citrate buffer (pH 6.0)

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Cell	Transfectant, HeLa, A549	NIH/3T3, WEHI-3B, L5178Y, C2C12	PC12	CHO
Reactivity	+	+ (weak)	+ (weak)	+ (weak)

Entrez Gene ID 4780 (Human)

REFERENCES

1) Taguchi, K., *et al.*, *Genes Cell* **16**, 123-140 (2011)
2) Komatsu, M., *et al.*, *Nat. Cell Biol.* **12**, 213-223 (2010)
3) Nguyen, T., *et al.*, *J. Biol. Chem.* **284**, 13291-13295 (2009)

For more information, please visit our web site **http://ruo.mbl.co.jp/**

MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
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RELATED PRODUCTS

Antibodies

PM069	Anti-NRF2 pAb	
M200-3	Anti-NRF2 mAb (1F2)	
M062-3	Anti-SOD1 (Human) mAb (1G2)	
M063-3	Anti-Thioredoxin (Human) mAb (2E3)	
M064-3	Anti-NADPH-Flavin Reductase mAb (2C10)	
PM036	Anti-LC3 pAb	[WB, IP, IC, IHC, FCM]
M152-3	Anti-LC3 mAb (4E12)	[WB, IP, IC, FCM, EM]
M186-3	Anti-LC3 mAb (8E10)	[WB]
PD014	Anti-LC3 pAb	[WB]
PD015	Anti-LC3 pAb	[IC]
PM046	Anti-LC3 pAb	[WB, IC]
M115-3	Anti-LC3 mAb (51-11)	[WB]
PM045	Anti-p62 (SQSTM1) pAb	
M162-3	Anti-p62 (SQSTM1) (Human) mAb (5F2)	
M162-A48	Anti-p62 (SQSTM1) (Human) mAb -Alexa Fluor®488 (5F2)	
M162-A59	Anti-p62 (SQSTM1) (Human) mAb -Alexa Fluor®594 (5F2)	
M162-A64	Anti-p62 (SQSTM1) (Human) mAb -Alexa Fluor®647 (5F2)	
PM066	Anti-p62 C-terminal pAb	
PD017	Anti-Beclin 1 pAb	
PM037	Anti-GABARAP pAb	
M135-3	Anti-GABARAP mAb (1F4)	
PM038	Anti-GATE-16 pAb	
PD041	Anti-Atg2A pAb	
PM034	Anti-Atg3 pAb	
M133-3	Anti-Atg3 mAb (3E8)	
M134-3	Anti-Atg4B mAb (9H5)	
PM050	Anti-Atg5 pAb	
M153-3	Anti-Atg5 mAb (4D3)	
PM039	Anti-Atg7 (Human) pAb	
PD042	Anti-Atg9A pAb	
M151-3	Anti-Atg10 (Human) mAb (5A7)	
M154-3	Anti-Atg12 (Human) mAb (6E5)	
PD036	Anti-Atg13 (Human) pAb	
M183-3	Anti-Atg13 mAb (5G4)	
PD026	Anti-Atg14 pAb	
M184-3	Anti-Atg14 (Human) mAb (4H8)	
PM040	Anti-Atg16L pAb	
M150-3	Anti-Atg16L mAb (1F12)	
M160-3	Anti-UVRAG mAb (1H4)	
PD027	Anti-Rubicon (Human) pAb	
M170-3	Anti-Rubicon (Human) mAb (1H6)	
PD037	Anti-Tel2 pAb	
PM072	Anti-VMP1 pAb	
M175-3	Anti-α-Tubulin mAb (2F9)	
M175-A48	Anti-α-Tubulin mAb-Alexa Fluor®488 (2F9)	
M175-A59	Anti-α-Tubulin mAb-Alexa Fluor®594 (2F9)	
M175-A64	Anti-α-Tubulin mAb-Alexa Fluor®647 (2F9)	
PM054	Anti-α-Tubulin pAb	
PM054-7	Anti-α-Tubulin pAb-HRP-DirecT	
M176-3	Anti-EEA1 mAb (3C10)	
M176-A48	Anti-EEA1 mAb-Alexa Fluor®488 (3C10)	
M176-A59	Anti-EEA1 mAb-Alexa Fluor®594 (3C10)	
M176-A64	Anti-EEA1 mAb-Alexa Fluor®647 (3C10)	
PM062	Anti-EEA1 pAb	
M178-3	Anti-Calnexin mAb (4F10)	
M178-A48	Anti-Calnexin mAb-Alexa Fluor®488 (4F10)	
M178-A59	Anti-Calnexin mAb-Alexa Fluor®594 (4F10)	
M178-A64	Anti-Calnexin mAb-Alexa Fluor®647 (4F10)	
PM060	Anti-Calnexin pAb	
M181-3	Anti-KDEL mAb (1D5)	
PM059	Anti-KDEL pAb	
M179-3	Anti-GM130 mAb (5G8)	
M179-A48	Anti-GM130 mAb-Alexa Fluor®488 (5G8)	
M179-A59	Anti-GM130 mAb-Alexa Fluor®594 (5G8)	
M179-A64	Anti-GM130 mAb-Alexa Fluor®647 (5G8)	
PM061	Anti-GM130 pAb	
PM063	Anti-COX4 pAb	
PM064	Anti-Lamin B1 pAb	

Kits

8485	Autophagy Ab Sampler Set
PM036-PN	Positive control for anti-LC3 antibody

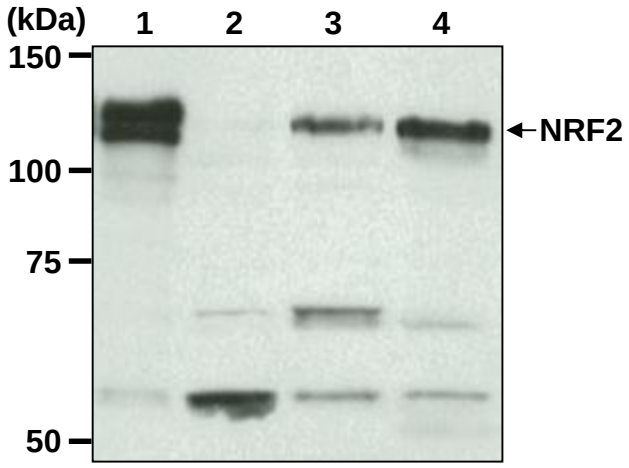
WB: Western blotting
IP: Immunoprecipitation
IC: Immunocytochemistry
IHC: Immunohistochemistry
FCM: Flow cytometry
EM: Immuno-electron microscopy

Other related antibodies and kits are also available.
Please visit our web site at http://ruo.mbl.co.jp

SDS-PAGE & Western blotting

1) Wash 1 x 10^7 cells 3 times with PBS and suspends them in 1 mL of Laemmli's sample buffer, then sonicate briefly (up to 20 sec.).
2) Boil the samples for 3 min. and centrifuge. Load 10 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (7.5% acrylamide) for electrophoresis.
3) 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.
4) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 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 **APPLICATIONS** 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 the 1:10,000 anti-IgG (Rabbit)-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 1 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; Trasnfectant, HeLa and A549)

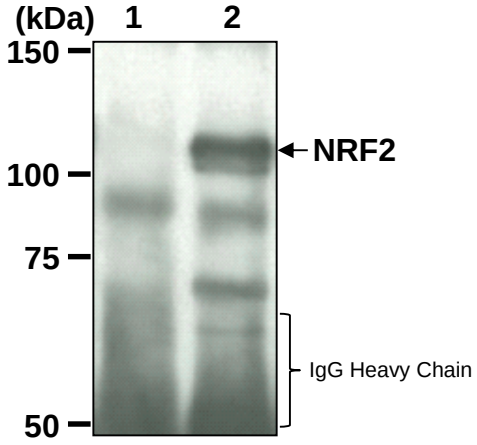


Western blot analysis of NRF2
Lane 1: NRF2/293T
Lane 2: 293T
Lane 3: HeLa
Lane 4: A549
Immunoblotted with PM069

Immunoprecipitation

1) Wash 1 x 10^7 cells 2 times with PBS and resuspend them with 1 mL of ice-cold Lysis buffer (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40) containing appropriate protease inhibitors, then sonicate briefly (up to 20 sec.).
2) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and transfer the supernatant to another tube.
3) Mix 20 µL of 50% protein A agarose beads slurry resuspended in 300 µL of IP buffer (10 mM Tris-HCl (pH 8.0), 500 mM NaCl, 0.1% NP-40) with primary antibody as suggested in the **APPLICATIONS**. Incubate with gently agitation for 1 hr. at room temperature.
4) Wash the beads 3 times with 1 mL of IP buffer.
5) Add 300 µL of cell lysate (prepared sample from step 2)), then incubate with gentle agitation for 1 hr. at room temperature.
6) Wash the beads 5 times with 1 mL of Lysis buffer.
7) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3 min. and centrifuge.
8) Load 10 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (7.5% acrylamide) for electrophoresis.
9) 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.
10) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for overnight at 4°C.
11) Wash the membrane with PBS-T (0.05% Tween-20 in PBS) (5 min. x 3 times).
12) Incubate the membrane with 1:1,000 anti-NRF2 (Human) pAb (MBL; code no. PM069) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
13) Wash the membrane with PBS-T (5 minutes x 3 times).
14) Incubate the membrane with the 1:10,000 anti-IgG (Rabbit)-HRP (MBL; code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
15) Wash the membrane with PBS-T (5 min. x 3 times).
16) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min.
17) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
18) Expose to an X-ray film in a dark room for 1 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; HeLa)

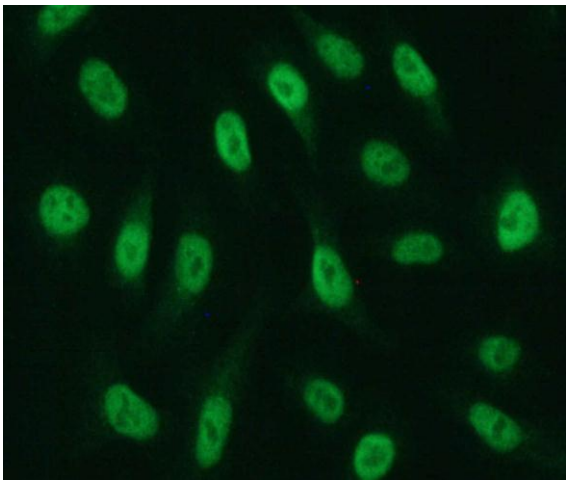


Immunoprecipitation of NRF2 from HeLa
Lane 1: IP with normal rabbit IgG (MBL; code no. PM035)
Lane 2: IP with PM069
Immunoblotted with PM069

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.
3) Wash the slide 2 times with PBS.
4) Fix the cells with 4% paraformaldehyde (PFA)/PBS for 10 min. at room temperature (20~25^{o}C).
5) Wash the slide 2 times with PBS.
6) Permeabilize the cells with 200 μL of 0.2% Triton X-100/PBS for 10 min. at room temperature.
7) Wash the slide 2 times with PBS.
8) Add 200 μL of the primary antibody diluted with 2% fetal calf serum (FCS)/PBS as suggested in the **APPLICATIONS** onto the cells and incubate for 1 hr. at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
9) Wash the slide 2 times with PBS.
10) Add 200 μL of 1:500 anti-IgG (Rabbit)-Alexa Fluor®488 (Invitrogen; code no. A110374) diluted with 2% fetal calf serum (FCS)/PBS onto the cells. Incubate for 30 min. at room temperature. Keep out light by aluminum foil.
11) Wash the slide 2 times with PBS.
12) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; HeLa)

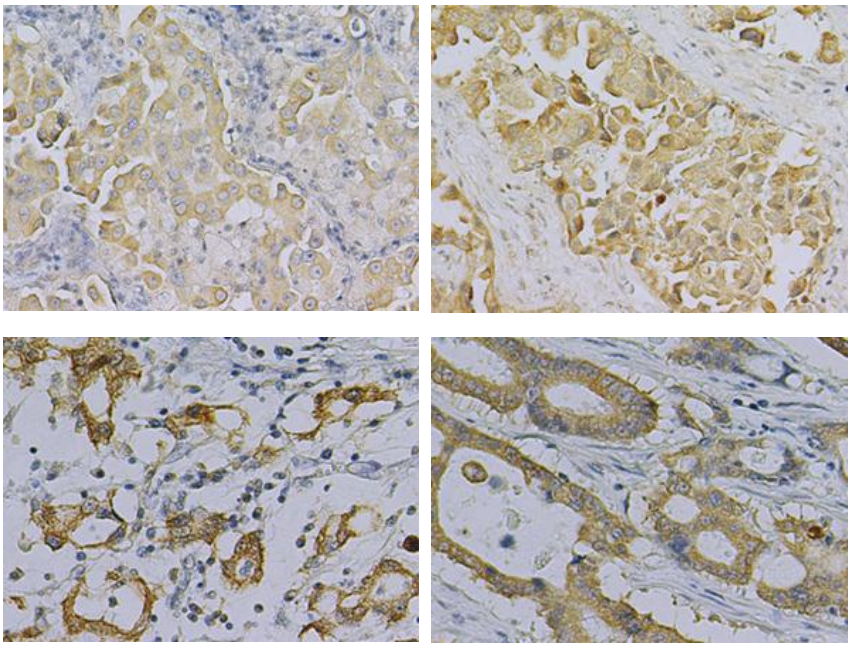


Immunocytochemical detection of NRF2 in HeLa
Green: PM069

Immunohistochemistry for formalin fixed paraffin-embedded section

1) Deparaffinize the sections with Xylene 3 times for 3 min. each.
2) Wash the slides with Ethanol 3 times for 3 min. each.
3) Wash the slides with PBS 3 times for 3 min. each.
4) Remove the slides from PBS and heat-treated 2 times with 10 mM Citrate buffer (pH6.0) for 10 min. each using microwave.
5) Let the slides cool down at room temperature in the Citrate buffer.
6) Wash the slides with running water for 5 min., then wash with PBS for 5 min.
7) Remove the slides from PBS and inactivate endogenous peroxidase with 3% H_2O_2 in PBS for 10 min.
8) Wash the slides with running water for 5 min., then wash with PBS for 5 min.
9) Remove the slides from PBS, wipe gently around each section and cover tissues with blocking buffer (20 mM HEPES/1% BSA/135 mM NaCl (pH 7.4)) for 5 min. at room temperature to block non-specific staining. Do not wash.
10) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with blocking buffer as suggest in the **APPLICATIONS**. (The concentration of antibody will depend on the conditions.) Incubate the sections for 1 hr. at room temperature.
11) Wash the slides 3 times in PBS for 5 min. each.
12) Wipe gently around each section and cover tissues with Histostar (Ms + Rb) (MBL; code no. 8460). Incubate for 30 min. at room temperature.
13) Wash the slides 3 times in PBS for 5 min. each.
14) Visualize by reacting for 10 min. with Histostar™ DAB Substrate Solution (MBL; code no. 8469). *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
15) Wash the slides in water for 5 min.
16) Counter stain in hematoxylin for 1 min., wash the slides 3 times in water for 5 min. each, and then immerse the slides in PBS for 5 min.
17) Dehydrate by immersing in Ethanol 3 times for 3 min. each, followed by immersing in Xylene 3 times for 3 min. each. Now ready for mounting.

(Positive controls for Immunohistochemistry; human lung carcinoma and human colon carcinoma)



Immunohistochemical detection of NRF2 in human cancer tissue

Upper: Lung carcinoma (different fields)
Lower: Colon carcinoma (different fields)