

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



Anti-NRF2 pAb

CODE No.	PM069
CLONALITY	Polyclonal
ISOTYPE	Rabbit Ig, affinity purified
QUANTITY	100 μ L
SOURCE	Purified Ig from rabbit serum
IMMUNOGEN	Human NRF2, full length (recombinant)
FORMULATION	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

<u>Western blotting</u>	1:1,000 for chemiluminescence detection system
<u>Immunoprecipitation</u>	5 μ L/300 μ L of cell extract from 3×10^6 cells
<u>Immunocytochemistry</u>	1:1,000
<u>Immunohistochemistry</u>	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/>

RELATED PRODUCTS

D299-3 Anti-IDH1-R132H (Human) mAb (HMab-1)
D300-3 Anti-IDH1-R132S (Human) mAb (SMab-1)
D309-3 Anti-IDH1 (Human) mAb (RMab-3)
D311-3 Anti-IDH2 (Human) mAb (RMab-22)
D328-3 Anti-IDH2-R172K (Human) mAb (KMab-1)
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]
PM045 Anti-p62 (SQSTM1) pAb
PM066 Anti-p62 C-terminal pAb
PM066-7 Anti-p62 C-terminal pAb-HRP-Direct
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)
PM074 Anti-Phospho-p62 (SQSTM1) (Ser351) pAb
M217-3 Anti-Phospho-p62 (SQSTM1) (Ser351) mAb (5D5)
D343-3 Anti-Phospho-p62 (SQSTM1) (Ser403) mAb (4F3)
D344-3 Anti-Phospho-p62 (SQSTM1) (Ser403) mAb (4C8)
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)
PM069 Anti-NRF2 pAb
M200-3 Anti-NRF2 mAb (1F2)
PD037 Anti-Tel2 pAb
PM072 Anti-VMP1 pAb
PM076 Anti-Syntaxin-17 (Human) pAb
M212-3 Anti-Syntaxin-17 (Human) mAb (2F8)
M224-3 Anti-KEAP1 mAb (KP1)
M230-3 Anti-Parkin mAb (Par6)
PM090 Anti-Atg8 (Filamentous fungi) pAb

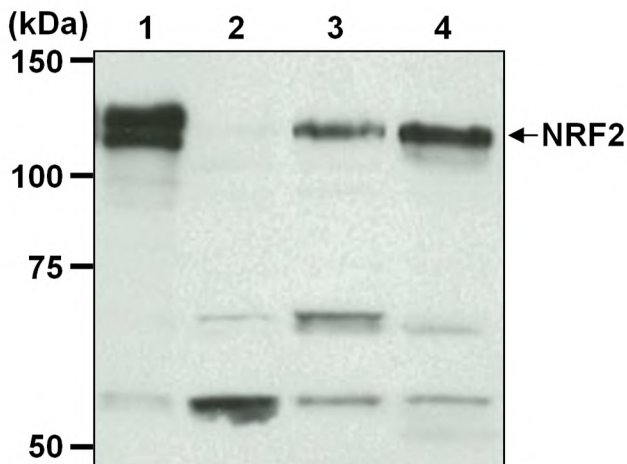
8485 Autophagy Ab Sampler Set
8486 Autophagy Watch
CY-7055 CycLex[®] Total p62 ELISA Kit
CY-7056 CycLex[®] Phospho-p62 Ser349 ELISA Kit
CY-7057 CycLex[®] Phospho-p62 Ser403 ELISA Kit
PM036-PN Positive control for anti-LC3 antibody

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

SDS-PAGE & Western blotting

- 1) Wash 1×10^7 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 μ L 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/cm² 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 **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 1:10,000 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 1 min. Develop the film as usual. The condition for exposure and development may vary.

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



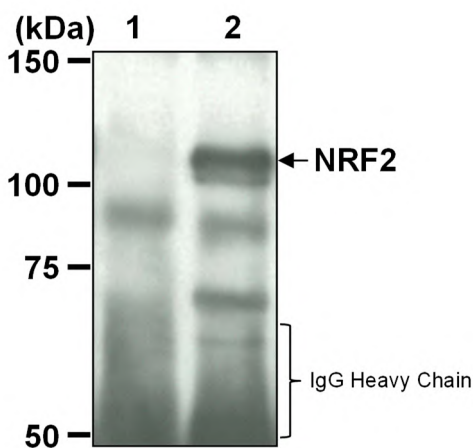
Western blot analysis of NRF2

Lane 1: NRF2/293T
Lane 2: 293T
Lane 3: HeLa
Lane 4: A549
Immunoblotted with PM069

Immunoprecipitation

- 1) Wash 1×10^7 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 μ L of 50% protein A agarose beads slurry resuspended in 300 μ L 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 μ L 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 μ L of Laemmli's sample buffer, boil for 3 min. and centrifuge.
- 8) Load 10 μ L 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/cm² 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) 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 1:10,000 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.
- 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)



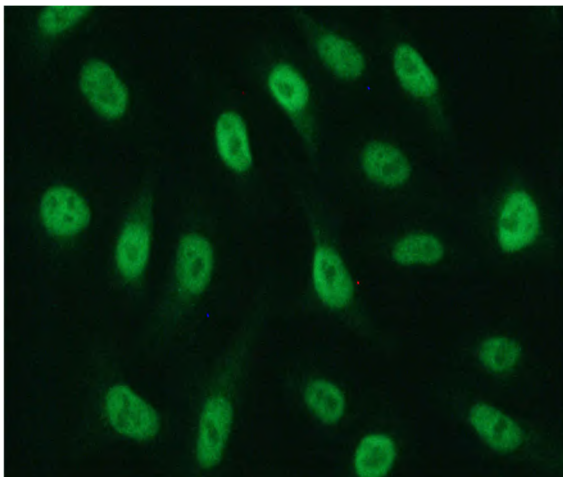
Immunoprecipitation of NRF2 from HeLa

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 CO₂ 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°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)



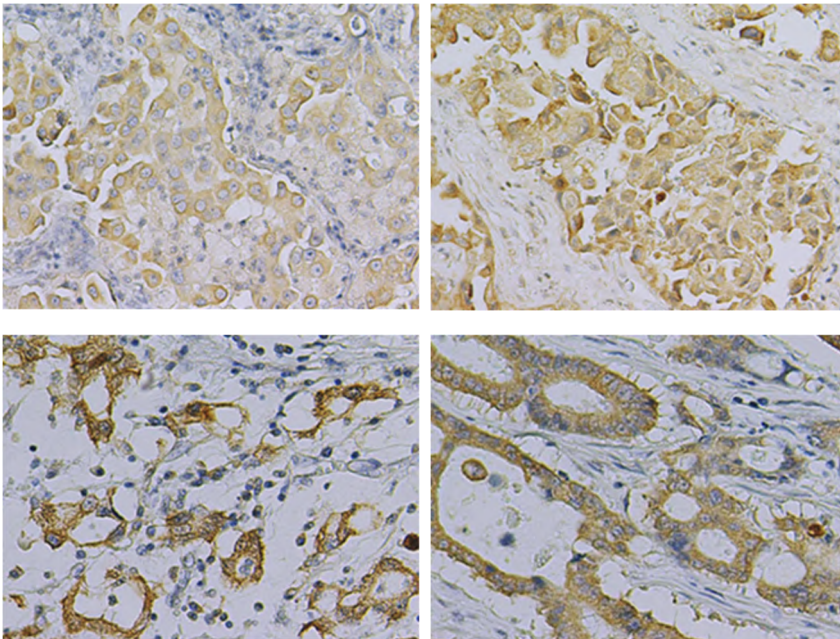
Immunocytochemical detection of NRF2 in HeLa

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% H₂O₂ 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)