**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 µL

**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

| | |
|---|---|
| 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/**

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## RELATED PRODUCTS

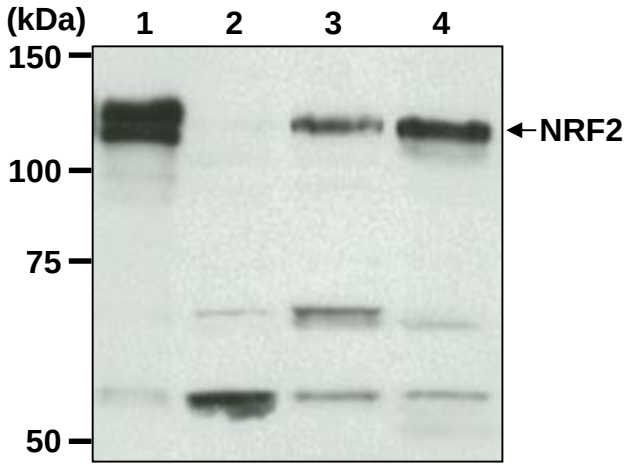
| | |
|---|---|
| D299-3 | Anti-IDH1-R132H (Human) mAb (HMab-1) |
| D300-3 | Anti-IDH1-R132S (Human) mAb (SMab-1) |
| D309-3 | Anti-IDH1 (Human) mAb (RMab-3) |
| D311-3 | Anti-IDH2 (Human) mAb (RMab-22) |
| D328-3 | Anti-IDH2-R172K (Human) mAb (KMab-1) |
| M062-3 | Anti-SOD1 (Human) mAb (1G2) |
| M063-3 | 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 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) 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; Trasnfectant, HeLa and A549)

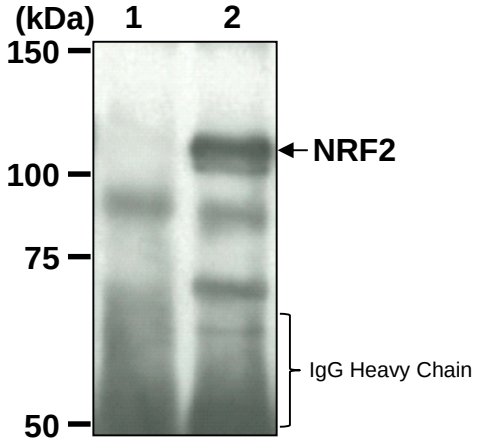



***Western blot analysis of NRF2***
Lane 1: NRF2/293T
Lane 2: 293T
Lane 3: HeLa
Lane 4: A549
Immunoblotted with PM069

## 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) 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)

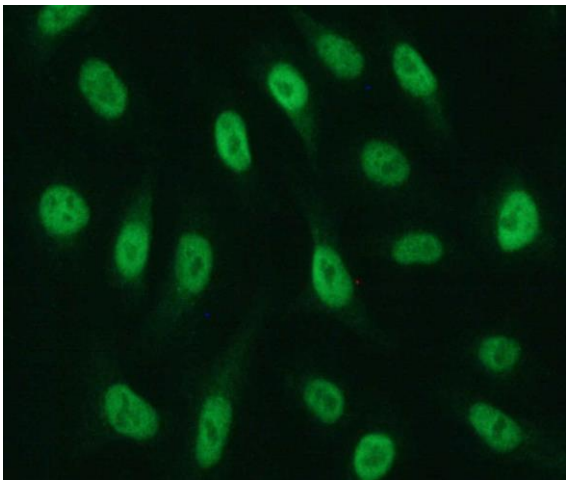



***Immunoprecipitation of NRF2 from HeLa***
Lane 1: IP with Normal rabbit IgG (MBL; code no. PM035)
Lane 2: IP with PM069
Immunoblotted with PM069

## Immunocytochemistry

1) Spread the cells on a glass slide, then incubate in a $CO_2$ 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)

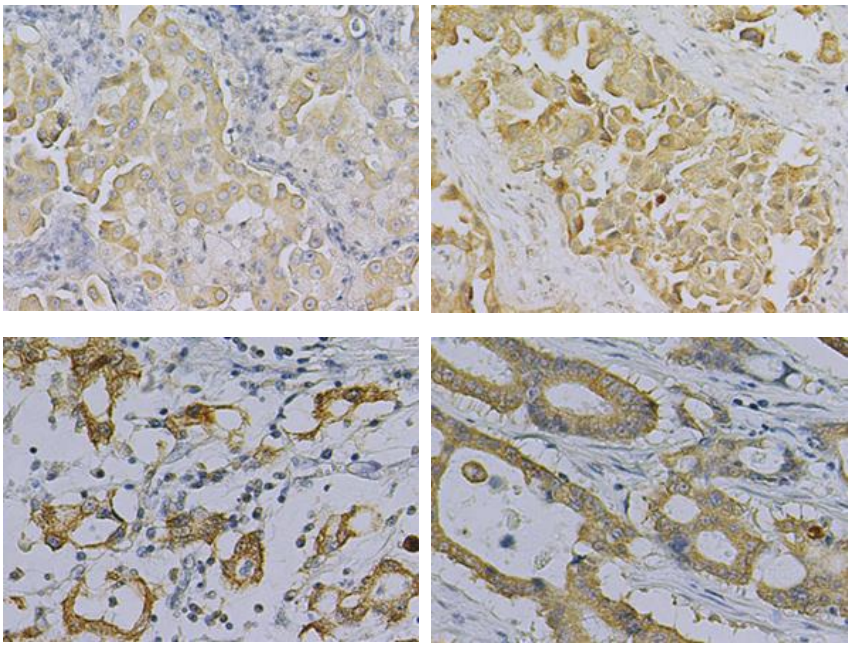


***Immunocytochemical detection of NRF2 in HeLa***
Green: PM069

## 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)