

 **My select** sampler set

Anti-NRF2 mAb

CODE No.	M200-3MS
CLONALITY	Monoclonal
CLONE	1F2
ISOTYPE	Mouse IgG1 κ
QUANTITY	20 μ L, 1 mg/mL
SOURCE	Purified IgG from hybridoma supernatant
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 μ g/mL
<u>Immunoprecipitation</u>	5 μ g/300 μ L of cell extract from 3×10^6 cells
<u>Immunocytochemistry</u>	0.5 μ g/mL
<u>Immunohistochemistry</u>	1 μ g/mL (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
Cells	Transfectant, HeLa	NIH/3T3	PC12	CHO
Reactivity	+	+	+	+

Entrez Gene ID 4780 (Human), 18024 (Mouse), 83619 (Rat), Hamster (100752102)

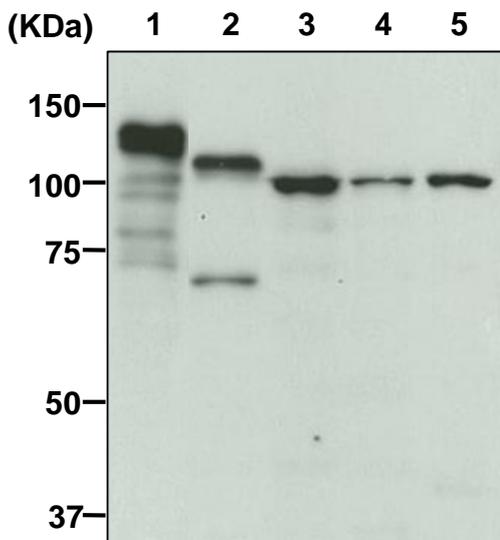
REFERENCE 1) Nguyen, T., *et al.*, *J. Biol. Chem.* **284**, 13291-13295 (2009)

For more information, please visit our web site <https://ruo.mbl.co.jp/>.

SDS-PAGE & Western blotting

- 1) Wash 1×10^7 cells 3 times with PBS and suspend 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% methanol). 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) 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.)
- 6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3).
- 7) Incubate the membrane with 1:10,000 of Anti-IgG (H+L chain) (Mouse) pAb-HRP (MBL, code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
- 8) Wash the membrane with PBS-T (5 min. x 3).
- 9) 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.
- 10) 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; Transfectant, HeLa, PC12, CHO, NIH/3T3)



Western blot analysis of NRF2

Lane 1: NRF2/293T

Lane 2: HeLa

Lane 3: PC12

Lane 4: CHO

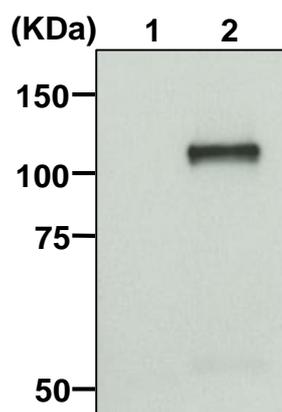
Lane 5: NIH/3T3

Immunoblotted with Anti-NRF2 mAb (MBL, code no. M200-3)

Immunoprecipitation

- 1) Wash 1×10^7 cells twice with PBS and resuspend them with 1 mL of ice-cold Extraction 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 gentle agitation for 1 hr. at 4°C.
- 4) Wash the beads once 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 4°C.
- 6) Wash the beads 4 times with 1 mL of Extraction 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% methanol). 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) Incubate the membrane with 1:1,000 of Anti-NRF2 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.)
- 12) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3).
- 13) Incubate the membrane with 1:10,000 of Anti-IgG (H+L chain) (Rabbit) pAb-HRP (MBL, code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
- 14) Wash the membrane with PBS-T (5 min. x 3).
- 15) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min.
- 16) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 17) 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: Isotype control (MBL, code no. M075-3)

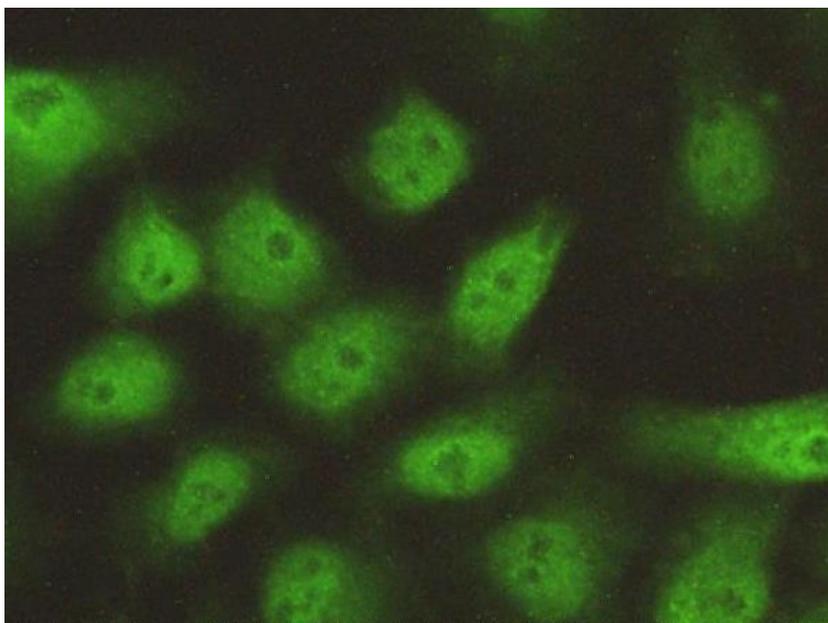
Lane 2: Anti-NRF2 mAb (MBL, code no. M200-3)

Immunoblotted with Anti-NRF2 pAb (MBL, code no. PM069)

Immunocytochemistry

- 1) Spread the cells on a glass slide, then incubate in a CO₂ incubator overnight.
- 2) Remove the culture supernatant by careful aspiration.
- 3) Wash the slide twice with PBS.
- 4) Fix the cells with 4% paraformaldehyde/PBS for 10 min. at room temperature (20~25°C).
- 5) Wash the slide twice 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 twice with PBS.
- 8) Add 200 µL of the primary antibody diluted with 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 twice with PBS.
- 10) Add 200 µL of 1:500 Alexa Fluor[®] 488 Goat Anti-mouse IgG (Thermo Fisher Scientific, code no. A11001) diluted with PBS onto the cells. Incubate for 30 min. at room temperature. Keep out light by aluminum foil.
- 11) Wash the slide twice with PBS.
- 12) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; HeLa)



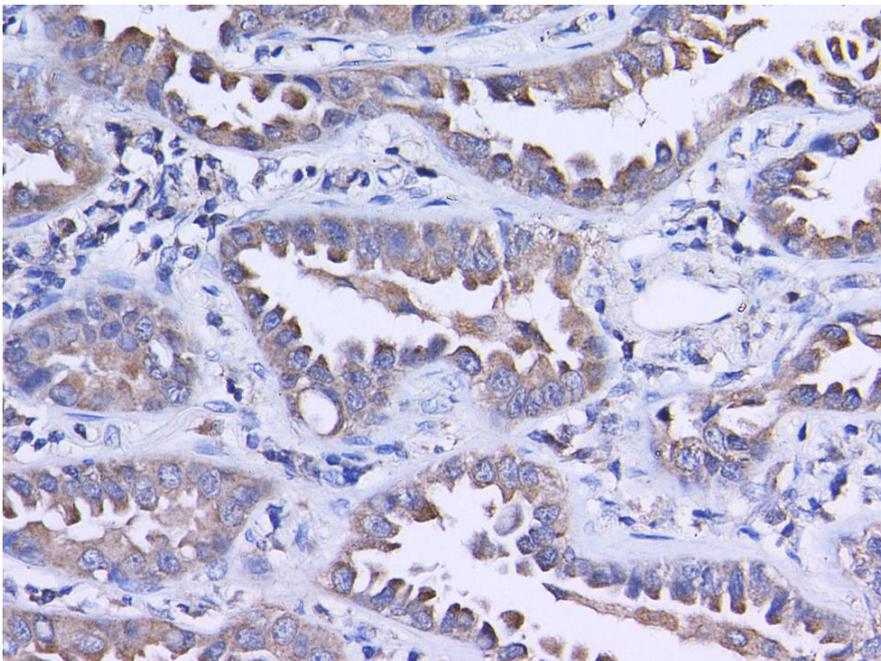
Immunocytochemical detection of NRF2 in HeLa

Green: Anti-NRF2 mAb (MBL, code no. M200-3)

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 twice with 10 mM Citrate buffer (pH 6.0) for 20 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 3 times in PBS for 5 min. each.
- 9) Remove the slides from PBS, wipe gently around each section and cover tissues with blocking buffer [20 mM HEPES (pH 7.4), 1% BSA, 135 mM NaCl] 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 suggested 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 1 hr. at room temperature.
- 13) Wash the slides 3 times in PBS for 5 min. each.
- 14) Visualize by reacting for 5 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)



Immunohistochemical detection of NRF2 in human lung carcinoma

Brown: Anti-NRF2 mAb (MBL, code no. M200-3)
Blue: Hematoxylin