

Anti-ATAD2 (ANCCA) (Human) mAb

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|--------------------|---|
| CODE No. | M226-3 |
| CLONALITY | Monoclonal |
| CLONE | C20-19 |
| ISOTYPE | Mouse IgG1 κ |
| QUANTITY | 100 μ L, 1 mg/mL |
| SOURCE | Purified IgG from hybridoma supernatant |
| IMMUNOGEN | Recombinant protein, corresponding to amino acids 1159-1350 of human ATAD2 (ANCCA) |
| 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

| | |
|-----------------------------|--|
| <u>Western blotting</u> | 1 μ g/mL for chemiluminescence detection system |
| <u>Immunoprecipitation</u> | 5 μ g/sample |
| <u>Immunohistochemistry</u> | 5 μ g/mL |
| | Heat treatment for paraffin embedded section: Autoclave; 120°C for 5min. in 10 mM Citrate buffer (pH 6.2). |
| <u>Immunocytochemistry</u> | 1 μ g/mL |

SPECIES CROSS REACTIVITY on WB

| Species | Human | Mouse | Rat | Monkey |
|------------|---------|---------|------|--------|
| Samples | SK-BR-3 | NIH/3T3 | Rat1 | COS-7 |
| Reactivity | + | - | - | - |

Entrez Gene ID 29028 (Human)

REFERENCES
1) Kalashnikova, E. V., *et al.*, *Cancer Res* **70**, 9402-9412 (2010)
2) Ciró, M., *et al.*, *Cancer Res* **69**, 8491-8498 (2009)

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

RELATED PRODUCTS

M226-3 Anti-ATAD2 (ANCCA) (Human) mAb

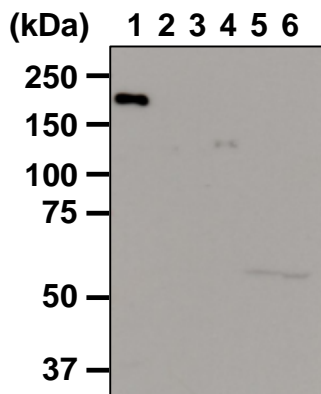
D318-3 Anti-Brm (Smarca2) mAb

D319-3 Anti-Brg1 (Smarca4) mAb

SDS-PAGE & Western blotting

- 1) Wash 5×10^6 cells 3 times with PBS and suspend them in 500 μ L of Laemmli's sample buffer, then sonicate briefly (up to 10 sec.). Centrifuge at 12,000 xg for 5 min. at 4°C.
- 2) Boil the sample for 10 min. and centrifuge.
- 3) Load 10 μ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (7.5% acrylamide) for electrophoresis.
- 4) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm² for 1 hr. in a dry transfer system.
- 5) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
- 6) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATION** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
- 7) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
- 8) Incubate the membrane with 1:5,000 of Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) 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, 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 control for Western blotting; SK-BR-3)



Western blot analysis of human ATAD2 (ANCCA)

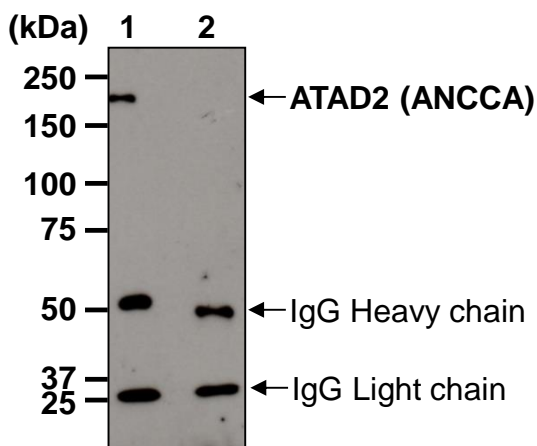
- Lane 1: SK-BR-3
- Lane 2: MDA-MB-231
- Lane 3: A431
- Lane 4: COS-7
- Lane 5: Rat1
- Lane 6: NIH/3T3

Immunoblotted with Anti-ATAD2 (ANCCA) (Human) mAb (M226-3)

Immunoprecipitation

- 1) Wash 5×10^6 cells 3 times with PBS and resuspend them with 500 μ L of RIPA Buffer [10 mM Tris-HCl (pH7.4), 1% Triton X-100, 1% Sodium deoxycholate, 0.1% SDS, 150 mM NaCl, 5 mM EDTA] containing appropriate protease inhibitors, then sonicate briefly (up to 30 sec.). Incubate on ice for 30 min.
- 2) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and transfer the supernatant to another tube.
- 3) Mix 50 μ L of 50% protein G agarose beads slurry resuspended in 500 μ L of PBS with Mouse IgG1 (isotype control) (MBL; code no. M075-3) or Anti-ATAD2 (ANCCA) (Human) mAb (M226-3) as suggested in the **APPLICATIONS**. Incubate at 4°C with rotation for 1 hr.
- 4) Wash the beads 3 times with PBS. Carefully discard the supernatant.
- 5) Add 5 μ L of the supernatant (prepared in step 4) and 400 μ L of IP Buffer [10 mM Tris-HCl (pH 8.0), 150 mM NaCl] to the tube containing antibody conjugated beads, then incubate with gentle agitation overnight at 4°C.
- 6) Wash 4 times with 1 mL of PBS-T [0.05% Tween-20 in PBS].
- 7) Resuspend the bead pellet in 50 μ L of Laemmli's sample buffer, boil for 10 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 dry transfer system.
- 10) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
- 11) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATION** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
- 12) Wash the membrane with PBS-T (5 min. x 3 times).
- 13) Incubate the membrane with 1:5,000 of Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) 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 times).
- 15) Wipe excess buffer on the membrane, 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.
- 16) 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; SK-BR-3)



Immunoprecipitation of human ATAD2 (ANCCA) from SK-BR-3

Lane 1: Anti-ATAD2 (ANCCA) (Human) mAb (M226-3)

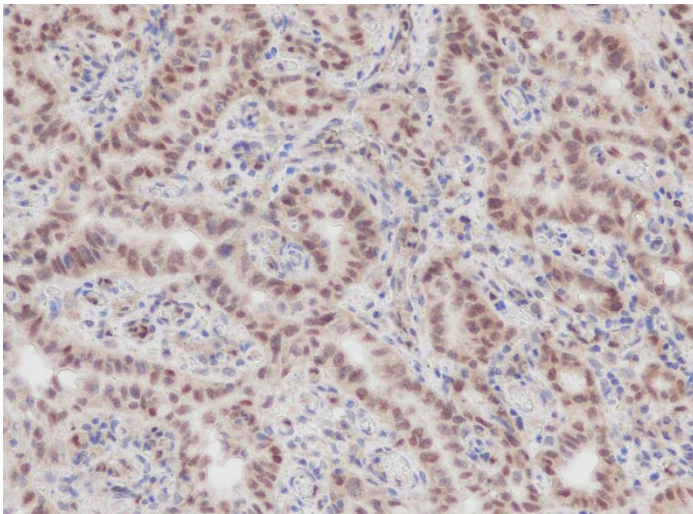
Lane 2: Mouse IgG1 (isotype control) (M075-3)

Immunoblotted with M226-3

Immunohistochemical detection for paraffin embedded section

- 1) Deparaffinize the section with Xylene 3 times for 5 min. each.
- 2) Immerse the slide with 100% Ethanol 2 times for 5 min. each.
- 3) Immerse the slide with 95% Ethanol 1 time for 5 min., and 70% Ethanol 1 time for 5 min.
- 4) Remove the slide from Ethanol and heat-treat with 10 mM citrate buffer (pH 6.2) at 121°C for 5 min. using autoclave.
- 5) Let the slide cool down at room temperature in citrate buffer.
- 6) Remove the slide from citrate buffer and inactivate endogenous peroxidase with 3% H₂O₂ in PBS for 10 min.
- 7) Wash the slide 3 times in PBS for 3 min. each.
- 8) Immerse the slide in 10% goat serum in PBS for 30 min. at room temperature to block non-specific staining.
- 9) Incubate the section with the primary antibody diluted with PBS containing 1% BSA as suggested in the **APPLICATIONS** overnight at 4°C. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
- 10) Wash the slides 3 times in PBS for 3 min. each.
- 11) Incubate the section with Histostar (Ms + Rb) (MBL; code no. 8460) for 30 min. at room temperature.
- 12) Wash the slides 3 times in PBS for 3 min. each.
- 13) Visualize by reacting for 3 min. with Histostar DAB Substrate Solution (MBL; code no. 8469). *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
- 14) Wash the slides in water for 5 min.
- 15) Counterstain in hematoxylin for 5 min., wash the slides 3 times in water for 5 min. each, and then immerse the slides in PBS for 5 min.
- 16) 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 control for Immunohistochemistry; Human specimen of stomach cancer)



Immunohistochemical detection of human ATAD2 (ANCCA)

Sample: Human specimen of stomach cancer
Brown: Anti-ATAD2 (ANCCA) (Human) mAb (M226-3)
Blue: Hematoxylin

Sample was kindly provided by Department of Pathology, Graduate School of Medicine, The University of Tokyo.

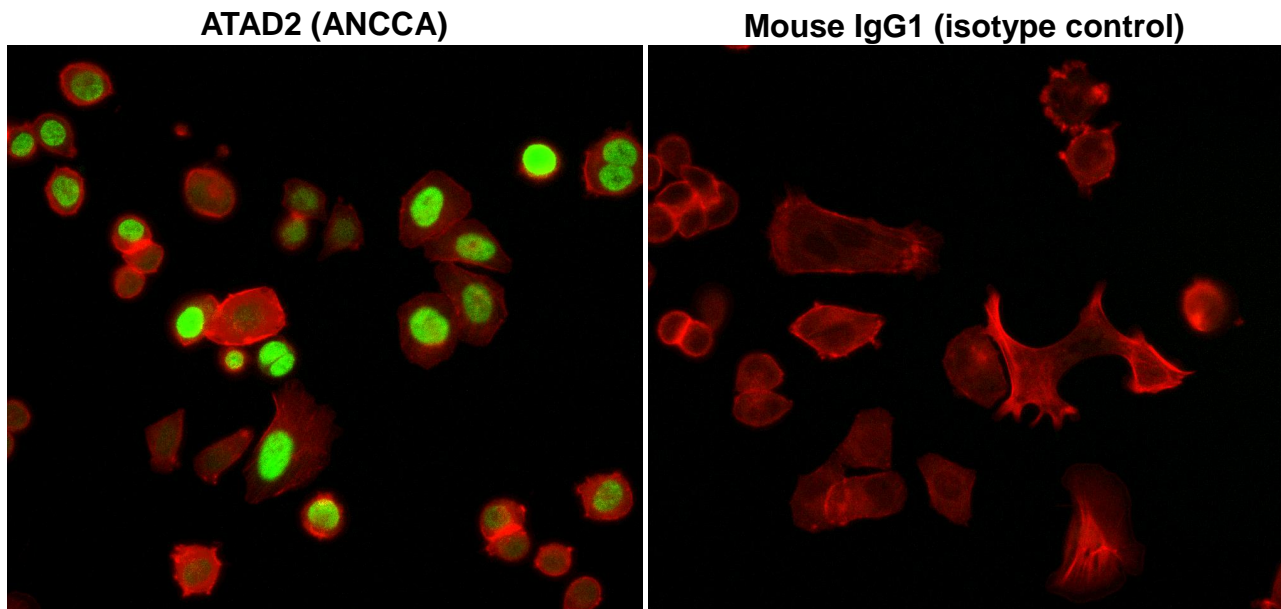
Data were kindly provided by Drs. Hiroto Katoh and Shumpei Ishikawa.

(Department of Genomic Pathology, Medical Research Institute, Tokyo Medical and Dental University)

Immunocytochemistry

- 1) Fix the cells by immersing the slide in 4% paraformaldehyde in PBS for 15min. at room temperature (20~25°C).
- 2) Wash the slide 3 times with PBS.
- 3) Permeabilize the cells with 0.5% Triton X-100 in PBS for 5 min. at room temperature.
- 4) Wash the slide 3 times with PBS.
- 5) Block the cells with 5% BSA in PBS for 30 min. at room temperature.
- 6) Incubate the cells with the primary antibody diluted with 1% BSA in PBS as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
- 7) Wash the slide 3 times with PBS.
- 8) Incubate the cells with 1:1,000 of Goat anti-Mouse IgG (H+L) Secondary Antibody, Alexa Fluor® 488 conjugate (Thermo Fisher Scientific; code no. A-11089) for 1 hr. at room temperature.
- 9) Wash the slide 3 times with PBS.
- 10) Counterstain with Acti-stain 555 phalloidin (Cytoskelton; code no. PHDH1) for 1 hr. at room temperature.
- 11) Now ready for mounting.

(Positive control for Immunocytochemistry; SK-BR-3)



Immunocytochemical detection of human ATAD2 (ANCCA)

Cells: SK-BR-3

Green: Anti-ATAD2 (ANCCA) (Human) mAb (M226-3)

Red: Acti-stain 555 phalloidin