

MONOCLONAL ANTIBODY

Anti-UVRAG

Code No.	Clone	Subclass	Quantity	Concentration
M160-3	1H4	Mouse IgG1 κ	100 μ g	1 mg/mL

BACKGROUND: Autophagy is a process of intracellular bulk degradation in which cytoplasmic components including organelles are sequestered within double-membrane vesicles that deliver the contents to the lysosome/vacuole for degradation. UVRAG (UV irradiation resistance-associated gene) has been identified as an essential component of the Beclin 1-PI3KC3 complex that suppresses tumorigenicity of human colon and promotes Beclin 1 dependent autophagy.

SOURCE: This antibody was purified from hybridoma (clone 1H4) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with C3H mouse lymphocyte immunized with the recombinant protein corresponding to amino acid residues 389 to 699 of human UVRAG.

FORMULATION: 100 μ g IgG in 100 μ L volume of 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 .

REACTIVITY: This antibody reacts with UVRAG on Western blotting.

APPLICATIONS:

Western blotting: 1 μ g/mL for chemiluminescence detection system

Immunoprecipitation: Not recommended

Immunohistochemistry: Not tested

Immunocytochemistry: Not tested

Flow cytometry: Not tested

Detailed procedure is provided in the following **PROTOCOL.**

SPECIES CROSS REACTIVITY:

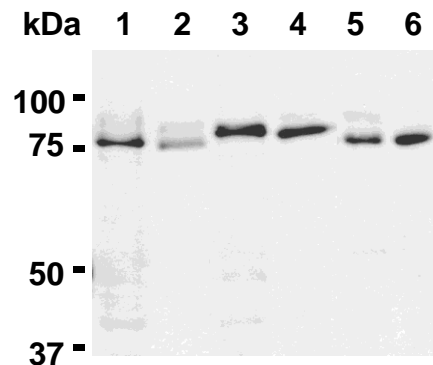
Species	Human	Mouse	Rat	Hamster
Cells	HeLa, Jurkat	NIH/3T3, WR19L	Rat1	CHO
Reactivity on WB	+	+	+	+

REFERENCES:

- 1) Liang, C., *et al.*, *Autophagy* **3**, 69-71 (2007)
- 2) Liang, C., *et al.*, *Nat. Cell Biol.* **8**, 688-699 (2006)

INTENDED USE:

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



Western blot analysis of UVRAG expression in HeLa (1), Jurkat (2), NIH/3T3 (3), WR19L (4), Rat1 (5) and CHO (6) using M160-3.

PROTOCOL:

SDS-PAGE & Western Blotting

- 1) Wash the 1×10^7 cells 3 times with PBS and suspend with 1 mL of Laemmli's sample buffer.
- 2) Boil the samples for 2 minutes and centrifuge. Load 10 μ L of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
- 3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm² for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
- 4) To reduce nonspecific binding, place 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 minutes x 3 times).
- 6) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
- 7) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).

- 8) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
- 9) Wash the membrane with PBS-T (5 minutes x 3 times).
- 10) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
- 11) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 12) Expose to an X-ray film in a dark room for 3 minutes.
- 13) Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; HeLa, Jurkat, NIH/3T3, WR19L, Rat1, CHO)

RELATED PRODUCTS:

PD017	anti-Beclin 1 (polyclonal)
PD014	anti-LC3 (polyclonal) (for WB)
PD015	anti-LC3 (polyclonal) (for IC)
PM036	anti-LC3 (polyclonal) (for WB, IP, FCM, IC, IHC)
PM046	anti-LC3 (polyclonal) (for WB, IC)
M115-3	anti-LC3 (51-11) (for WB)
M152-3	anti-LC3 (4E12) (for WB, IP, FCM, IC)
M135-3	anti-GABARAP (1F4)
PM037	anti-GABARAP (polyclonal)
PM038	anti-GATE-16 (polyclonal)
PM034	anti-Atg3 (polyclonal)
M133-3	anti-Atg3 (3E8)
M134-3	anti-Atg4B (9H5)
M153-3	anti-Atg5 (4D3)
PM050	anti-Atg5 (polyclonal)
PM039	anti-Atg7 (polyclonal)
M151-3	anti-Atg10 (5A7)
M154-3	anti-Atg12 (6E5)
PD036	anti-Atg13 (polyclonal)
PD026	anti-Atg14 (polyclonal)
PM040	anti-Atg16L (polyclonal)
M150-3	anti-Atg16L (1F12)
M162-3	anti-p62/SQSTM1 (5F2)
PM045	anti-p62/SQSTM1 (polyclonal)
M170-3	anti-Rubicon (1H6)
PD027	anti-Rubicon (polyclonal)
PM036-P	Positive control for anti-LC3 antibody

WB: Western blotting

IP: Immunoprecipitation

FCM: Flow cytometry

IC: Immunocytochemistry

IHC: Immunohistochemistry