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

Anti-Atg8 (Filamentous fungi) pAb

CODE No. PM090

CLONALITY Polyclonal
ISOTYPE Rabbit Ig, affinity purified
QUANTITY 100 µL

SOURCE Purified Ig from rabbit serum
IMMUNOGEN Recombinant protein, corresponding to amino acids 1-116 of rice blast fungus MGG_01062 (Atg8)
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.

APPLICATION-CONFIRMED
Western blotting 1:1,000

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Filamentous fungi
Sample	Not tested	Not tested	Not tested	*Aspergillus oryzae* strain NSRku70-1-1A
Reactivity				+

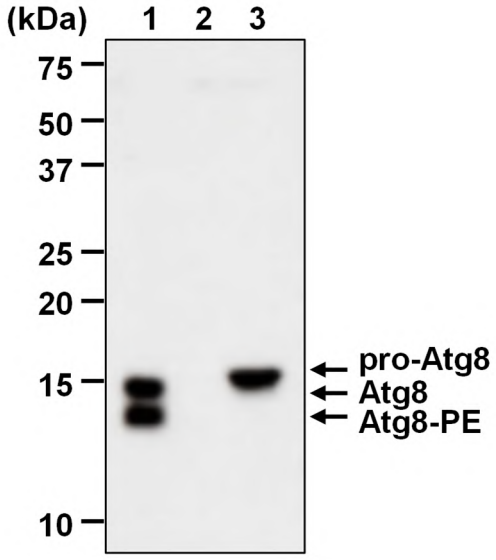
For more information, please visit our website at https://ruo.mbl.co.jp/.

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

SDS-PAGE & Western blotting

1) Boil the samples for 2 min. and centrifuge.
2) Load 4 μL (10 μg) of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (15% 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% methanol). See the manufacturer's manual for precise transfer procedure.
4) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 2 hr. at room temperature.
5) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3).
6) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** overnight at 4°C. (The concentration of antibody will depend on the conditions.)
7) Wash the membrane with PBS-T (5 min. x 3).
8) Incubate the membrane with 1:10,000 of 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).
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 40 sec. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; *Aspergillus oryzae* strain NSRku70-1-1A)



Western blot analysis of Aspergillus oryzae Atg8
Lane 1: WT (NSRku70-1-1A)
Lane 2: Disrupted Atg8
Lane 3: Disrupted Atg4

Immunoblotted with Anti-Atg8 (Filamentous fungi) pAb (PM090)

The samples were kindly provided by Dr. Takashi Kikuma.
(The University of Tokyo, Graduate School of Agricultural and Life Sciences Department of Biotechnology)