

# Anti-Atg8 (Filamentous fungi) pAb

<b>CODE No.</b>	PM090
<b>CLONALITY</b>	Polyclonal
<b>ISOTYPE</b>	Rabbit Ig, affinity purified
<b>QUANTITY</b>	100 µL
<b>SOURCE</b>	Purified Ig from rabbit serum
<b>IMMUNOGEN</b>	Recombinant protein, corresponding to amino acids 1-116 of rice blast fungus MGG_01062 (Atg8)
<b>FORMULATION</b>	PBS containing 50% Glycerol (pH 7.2). No preservative is contained.
<b>STORAGE</b>	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	<i>Aspergillus oryzae</i> strain NSRku70-1-1A
Reactivity				+

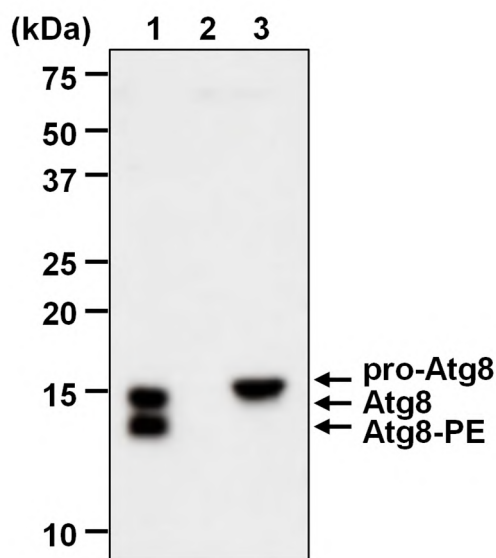
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The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

### **SDS-PAGE & Western blotting**

- 1) Boil the samples for 2 min. and centrifuge.
- 2) Load 4  $\mu$ L (10  $\mu$ g) 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<sup>2</sup> 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.  
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