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**Not for use in diagnostic procedures.**

## Anti-OsTIR1 pAb

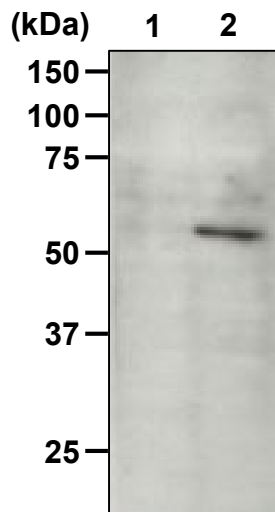
<b>CODE No.</b>	PD048
<b>CLONALITY</b>	Polyclonal
<b>ISOTYPE</b>	Rabbit IgG
<b>QUANTITY</b>	100 µL
<b>SOURCE</b>	Purified IgG from rabbit serum
<b>IMMUNOGEN</b>	Recombinant full-length <i>Oryza sativa</i> Transport inhibitor response 1 (OsTIR1) protein
<b>REACTIVITY</b>	This antibody reacts with <i>Oryza sativa</i> Transport inhibitor response 1 (OsTIR1).
<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.
<b>APPLICATION-CONFIRMED</b>	
<u>Western blotting</u>	1:1,000
<b>APPLICATION-REPORTED</b>	
<u>Immunohistochemistry</u>	Reference 2)
<b>REFERENCE</b>	1) Natsume, T., <i>et al.</i> , <i>Cell Rep.</i> <b>15</b> , 210-218 (2016) [WB] 2) Yesbolatova A <i>et al.</i> , <i>Nat Commun.</i> <b>11</b> , 5701 (2020) [IC]

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

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

- 1) Culture the cells in the appropriate condition on a 6-well plate until they reach about 90% confluence.
- 2) Wash the cells with PBS and resuspend them with 50  $\mu$ L of RIPA buffer [25 mM Tris-HCl (pH 7.6), 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS]. Transfer the lysate to a tube. Incubate on ice for 30 min.
- 3) Centrifuge the tube at 12,000 xg for 20 min. and transfer the supernatant to another tube.
- 4) Mix the supernatant with equal volume of Laemmli's sample buffer.
- 5) Boil the samples for 5 min. and centrifuge. Load 5  $\mu$ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (10% acrylamide) for electrophoresis.
- 6) 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.
- 7) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
- 8) Incubate the membrane with primary antibody diluted with 5% 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.)
- 9) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3).
- 10) Incubate the membrane with the 1:10,000 Anti-IgG (H+L chain) (Rabbit) pAb-HRP (MBL; code no. 458) diluted with 5% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
- 11) Wash the membrane with PBS-T (5 min. x 3)
- 12) 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.
- 13) 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.



#### ***Western blotting analysis of OsTIR1***

Sample: HCT116 Tet-OsTIR1

Lane 1: Untreated

Lane 2: Treated with 0.2  $\mu$ g/ $\mu$ L of doxycycline for 24 h

Immunoblotted with Anti-OsTIR1 pAb (PD048)

Sample was kindly provided by Dr. Masato Kanemaki. (National Institute of Genetics)