

## Anti-His-tag mAb

Code No.	Clone	Subclass	Quantity	Concentration
D291-3S	OGHis	Mouse IgG2a $\kappa$	50 $\mu$ L	1 mg/mL

**BACKGROUND:** The His-tag (6xHis-tag) is one of the most common tags used to facilitate the purification of recombinant proteins. Metal chelate affinity chromatography is widely used for purification of His-tagged proteins. This specific antibody is useful tool for monitoring of the His-tagged proteins, and recognizes His-tags placed at N-terminal, C-terminal, and internal regions of the recombinant proteins.

**SOURCE:** This antibody was purified from hybridoma (clone OGHIS) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell SP-1 with Balb/c mouse splenocyte immunized with 6xHis tagged protein.

**FORMULATION:** 50  $\mu$ g IgG in 50  $\mu$ 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  $-20^{\circ}\text{C}$ .

**REACTIVITY:** This antibody recognizes His-tagged protein on Western blotting, Immunoprecipitation, Immunocytochemistry and Flow cytometry.

### APPLICATIONS:

Western blotting: 0.2  $\mu$ g/mL

Immunoprecipitation: 1  $\mu$ g/sample

Immunohistochemistry: Not tested

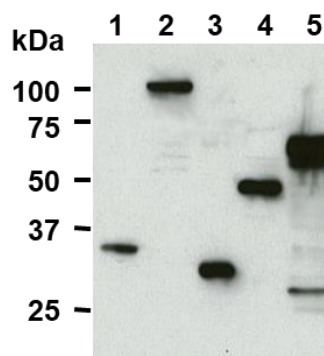
Immunocytochemistry: 0.5  $\mu$ g/mL

Flow cytometry: 0.2  $\mu$ g/mL (final concentration)

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

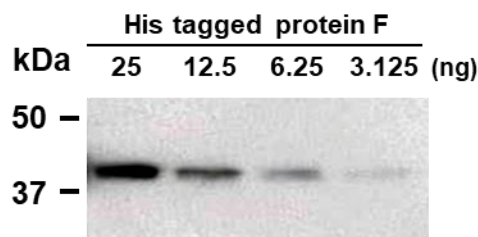
### INTENDED USE:

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



### Western blotting analysis of His-tagged protein using D291-3S.

Lane1: N-terminal Met-His-tagged protein A expressed in *E. coli*  
Lane2: Internal His-tagged protein B expressed in 293T  
Lane3: Internal His-tagged protein C expressed in *E. coli*  
Lane4: C-terminal His-tagged protein D expressed in 293T  
Lane5: C-terminal His-tagged protein E expressed in 293T



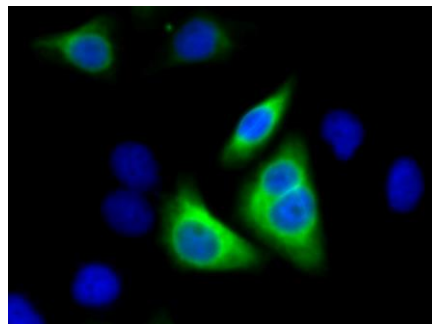
### Western blotting analysis of His-tagged protein F using D291-3S.

### PROTOCOLS:

#### SDS-PAGE & Western blotting

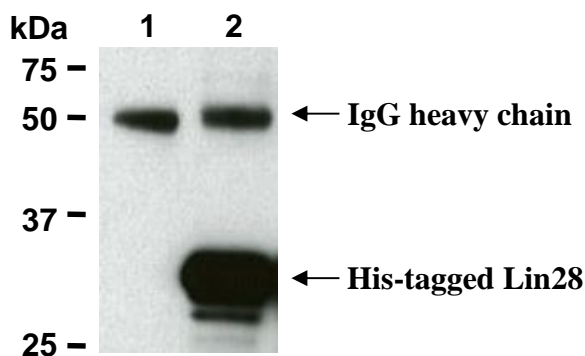
- 1) Mix the sample with equal volume of Laemmli's sample buffer.
- 2) Boil the samples for 3 minutes and centrifuge. Load 10  $\mu$ L of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
- 3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm<sup>2</sup> for 1 hour 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 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at  $4^{\circ}\text{C}$ .
- 5) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)

- 6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
  - 7) Incubate the membrane with 1:10,000 of Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
  - 8) Wash the membrane with PBS-T (5 minutes x 3 times).
  - 9) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
  - 10) Expose to an X-ray film in a dark room for 3 minutes. Develop the film as usual. The condition for exposure and development may vary.
- 7) Resuspend the beads in 20  $\mu$ L of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10  $\mu$ L/lane for the SDS-PAGE analysis.  
(See **SDS-PAGE & Western blotting**.)



**Immunocytochemical detection of His-tagged calnexin expressed in HeLa using D291-3S.**

Green: Alexa Fluor<sup>®</sup> 488  
Blue: DAPI



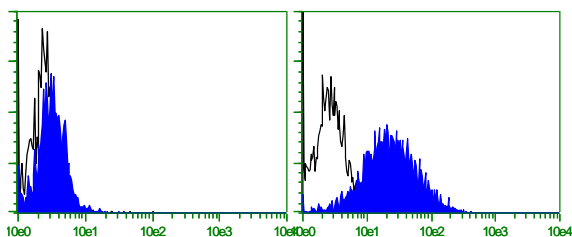
**Immunoprecipitation of His-tagged Lin28 expressed in 293T with Mouse IgG2a isotype control (1) or D291-3 (2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with D291-3.**

### **Immunoprecipitation**

- 1) Wash cells (approximately  $1 \times 10^7$  cells) 3 times with PBS and resuspend them in 1 mL of cold Lysis buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.05% NP-40) containing protease inhibitors at appropriate concentrations. Incubate it at 4°C with rotating for 30 minutes; thereafter, briefly sonicate the mixture (up to 10 seconds).
- 2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another fresh tube.
- 3) Add primary antibody as suggested in the **APPLICATIONS** into 300  $\mu$ L of the supernatant. Mix well and incubate with gentle agitation for 60-120 minutes at 4°C. Add 20  $\mu$ L of 50% protein A agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
- 4) Centrifuge the tube at 2,000 x g for 1 minute, and carefully discard the supernatant using a pipettor without disturbing the beads.
- 5) Resuspend the beads with cold Lysis Buffer.
- 6) Centrifuge the tube at 2,000 x g for 1 minute, and carefully discard the supernatant.

### **Immunocytochemistry**

- 1) Culture the cells in the appropriate condition on a glass slide. (For example, spread  $1 \times 10^4$  cells of transfectant cells for one slide, then incubate in a CO<sub>2</sub> incubator overnight.)
- 2) Wash the cells 3 times with PBS.
- 3) Fix the cells by immersing the slide in PBS containing 4% paraformaldehyde for 10 minutes at room temperature.
- 4) Wash the glass slide 3 times with PBS.
- 5) Immerse the slide in PBS containing 0.2% Triton X-100 for 10 minutes at room temperature.
- 6) Wash the glass slide 3 times with PBS.
- 7) Add the primary antibody diluted with PBS containing 2% FCS as suggested in the **APPLICATIONS** onto the cells and incubate for 60 minutes at room temperature (Optimization of antibody concentration or incubation condition are recommended if necessary.)
- 8) Wash the glass slide 2 times with PBS.
- 9) Add 100  $\mu$ L of 1:500 Alexa Fluor<sup>®</sup> 488 conjugated anti-mouse IgG (Thermo Fisher; code no. A11001) diluted with PBS containing 2% FCS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
- 10) Wash the glass slide 3 times with PBS.
- 11) Wipe excess liquid off the slide but take care not to touch the cells. Never leave the cells to dry.
- 12) Promptly add mounting medium onto the slide, then put a cover slip on it.



**Flow cytometric analysis of 293T (left) and His-tagged LAMP1 expressed in 293T (right).** Open histograms indicate the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of D291-3S to the cells.

### **Flow cytometric analysis for floating cells**

We usually use Fisher tubes or equivalents as reaction tubes for all steps described below.

- 1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.09% NaN<sub>3</sub>].  
\*Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.
- 2) Add 100  $\mu$ L of 4% paraformaldehyde (PFA) to the cell pellet after tapping. Mix well, then fix the cells for 10 minutes at room temperature.
- 3) Wash the cells twice with washing buffer.
- 4) Add 100  $\mu$ L of PBS containing 0.2% Triton X-100 to the cell pellet after tapping. Mix well, then permeabilize the cells for 10 minutes at room temperature.
- 5) Wash the cells once with washing buffer.
- 6) Add the primary antibody diluted with washing buffer as suggested in the **APPLICATIONS** onto the cells and incubate for 30 minutes at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
- 7) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 8) Add PE conjugated anti-mouse IgG antibody diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
- 9) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 10) Resuspend the cells with 500  $\mu$ L of the washing buffer and analyze by a flow cytometer.

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