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

My select sampler set

Anti-His-tag mAb

Code No.	Clone	Subclass	Quantity	Concentration
D291-3MS	OGHis	Mouse IgG2a κ	20 µL	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: 20 µg IgG in 20 µL 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 recognizes His-tagged protein on Western blotting, Immunoprecipitation, Immunocytochemistry and Flow cytometry.

APPLICATIONS:

Western blotting; 0.2 µg/mL
Immunoprecipitation; 1 µg/sample
Immunohistochemistry; Not tested
Immunocytochemistry; 0.5 µg/mL
Flow cytometry; 0.2 µg/mL (final concentration)

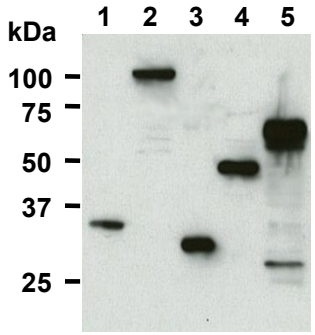
Detailed procedure is provided in the following **PROTOCOLS**.

REFERENCES:

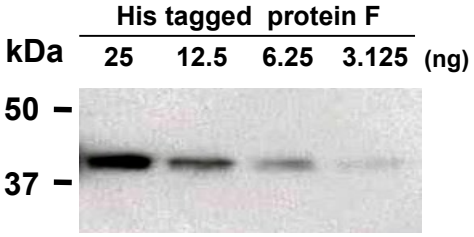
1) Cannavo, E., *et al., Nat. Commun.* **9**, 4016 (2018) [WB]
2) Teratani, T., *et al., J. Clin. Invest.* **128**, 1581-1596 (2018) [WB]
3) Ahsan, K. B., *et al., Sci. Rep.* **7**, 10446 (2017) [WB]
4) Ji, Y., *et al., Nat. Commun.* **8**, 15308 (2017) [WB]
5) Phadngam, S., *et al., Oncotarget* **7**, 84999-85020 (2016) [WB, IP, IC]
6) Bai, D., *et al., Nat. Commun.* **7**, 12310 (2016) [WB]
7) Ju, Y., *et al., Plant Cell* **28**, 2131-2146 (2016) [WB]
8) Han, J., *et al., Nature* **524**, 243-246 (2015) [WB]

INTENDED USE:

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



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

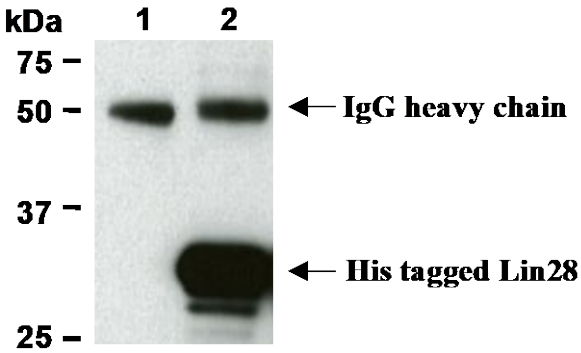
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

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 µL 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^2 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.

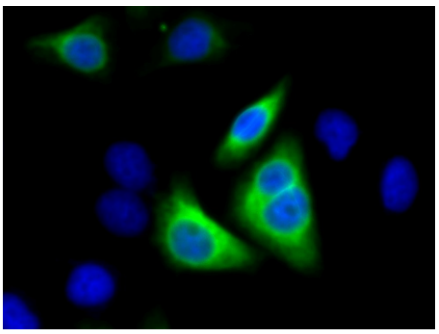
4) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
5) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hour at room temperature. (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).
7) Incubate the membrane with 1:10,000 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).
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.



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 x 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 µL of the supernatant. Mix well and incubate with gentle agitation for 60-120 minutes at 4°C. Add 20 µL 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 2,500 x g for 10 seconds and discard the supernatant.
5) Resuspend the agarose with cold Lysis buffer.
6) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
7) Repeat steps 5)-6) 2-4 times.
8) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10 µL/lane for the SDS-PAGE analysis.
(See **SDS-PAGE & Western blotting**.)



Immunocytochemical detection of His-tagged calnexin expressed in HeLa using D291-3.
Green: Alexa Fluor® 488
Blue: DAPI

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (For example, spread 1 x 10^4 cells of transfectant cells for one slide, then incubate in a CO_2 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 twice with PBS.
9) Add 100 µL of 1:500 Alexa Fluor® 488 conjugated anti-mouse IgG (Thermo Fisher Scientific; code no. A-11001) 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 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_3].
   *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 µL 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 µL 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 are 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 µL of the washing buffer and analyze by a flow cytometer.

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