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

MBL
A JSR Life Sciences Company

MONOCLONAL ANTIBODY

# Anti-His-tag mAb-Alexa Fluor® 647

| Code No. | Clone | Subclass | Quantity | Concentration |
|---|---|---|---|---|
| D291-A64 | OGHis | Mouse IgG2a κ | 50 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:** 50 µg of IgG in 50 µL volume of PBS containing 1% BSA and 0.1% ProClin 150.

**STORAGE:** This antibody solution is stable for one year from the date of purchase when stored at 4°C.

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

**APPLICATIONS:**
Immunocytochemistry; 1 µg/mL
Flow cytometry; 0.5 µg/mL (final concentration)
*Please refer to the data sheet (MBL code no. D291-3) for other applications.

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

**INTENDED USE:**
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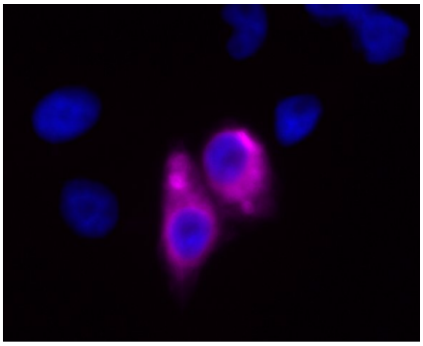
**PROTOCOLS:**

**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 for one night.)
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 30 minutes at room temperature (Optimization of antibody concentration or incubation condition is recommended if necessary.)
8) Wash the glass slide 2 times with PBS.
9) Counter stain with DAPI for 5 minutes at room temperature.
10) Wash the glass slide 2 times with PBS.
11) Wipe excess buffer 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.

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



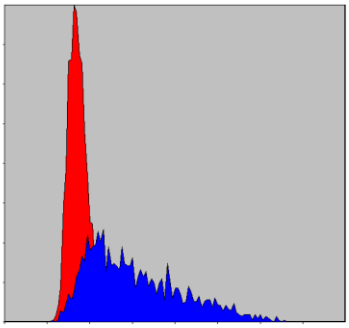
***Immunocytochemical detection of His-tagged calnexin expressed in HeLa using D291-A64.***
Magenta : Alexa Fluor® 647
Blue: DAPI

**Flow cytometric analysis for adherent cells**

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

1) Detach the cells from culture dish.
2) 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.
3) 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.
4) Wash the cells 2 times with washing buffer.

5) 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.
6) Wash the cells 1 time with washing buffer.
7) 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.)
8) 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.
9) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.



***Flow cytometric analysis of His-tagged calnexin expressed in 293T.*** *Red histogram indicates the reaction of isotypic control to the cells. Blue histogram indicates the reaction of D291-A64 to the cells.*

## RELATED PRODUCTS:

Other related antibodies and kits are also available.
Please visit our website at https://ruo.mbl.co.jp/