

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:

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

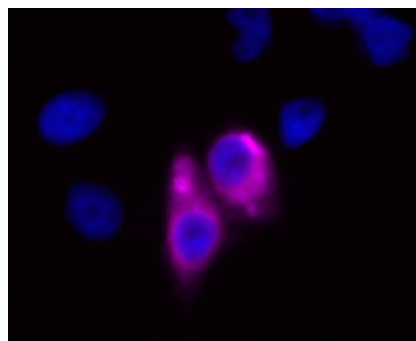
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

PROTOCOLS:

Immunocytochemistry

- 1) Culture the cells in the appropriate condition on a glass slide. (for example, spread 1×10^4 cells of transfectant cells for one slide, then incubate in a CO₂ 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.



Immunocytochemical detection of His-tagged calnexin expressed in HeLa using D291-A64.

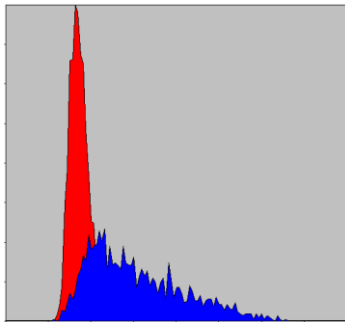
Magenta : Alexa Fluor® 647
Blue: DAPI

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% NaN₃].
*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 μ 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.
- 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 μ L 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/>

LABEL LICENSES:

This product is provided under an agreement between LIFE TECHNOLOGIES Corporation, and Medical & Biological Laboratories Co., LTD.

Alexa Fluor® is a registered trademark of Molecular Probes, Inc.