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

MBL
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MONOCLONAL ANTIBODY

Anti-His-tag mAb-Alexa Fluor® 594

Code No.	Clone	Subclass	Quantity	Concentration
D291-A59	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% ProClin150.

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.

APPLICATION:
Immunocytochemistry; 1 µg/mL
*Please refer to the data sheet (MBL, code no. D291-3) for other applications.

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

INTENDED USE:
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REFERENCE:
1) Ungerer, C., *et al.*, *Stem Cells Dev.* **23**, 755-766 (2014) [IC]

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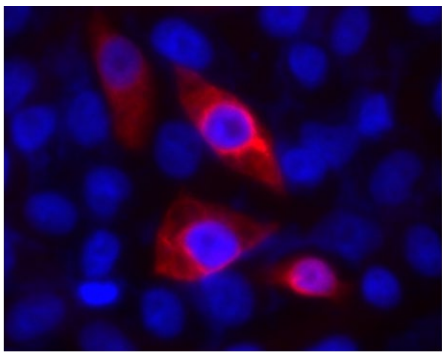

Immunocytochemical detection of His-tagged calnexin expressed in HeLa using D291-A59.
Green: Anti-His-tag mAb-Alexa Fluor® 594 (MBL, code no. D291-A59)
Blue: DAPI

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

PROTOCOL:

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 **APPLICATION** 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 twice with PBS.
9) Counter stain with DAPI for 5 minutes at room temperature.
10) Wash the glass slide twice 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.

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