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

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MONOCLONAL ANTIBODY

Anti-Myc-tag mAb-Alexa Fluor® 647

Code No.	Clone	Subclass	Quantity	Concentration
M047-A64	PL14	Mouse IgG1	100 µL	1 mg/mL

BACKGROUND: Epitope tagging has widely been accepted technique that fuses an epitope peptide to a certain protein as a marker for gene expression. With this technique, the gene expression can be easily monitored on western blotting, immunoprecipitation and immunofluorescence utilizing with an antibody that recognizes such an epitope. Amino acid sequences that are widely used for the epitope tagging are as follow; YPYDVPDYA (HA-tag), EQKLISEEDL (Myc-tag) and YTDIEMNRLGK (VSV-G-tag), which corresponding to the partial peptide of Influenza hemagglutinin protein, human c-myc gene product and Vesicular stomatitis virus glycoprotein respectively.

SOURCE: This antibody was purified from mouse ascites fluid using protein A agarose. This hybridoma (clone PL14) was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse splenocyte immunized with 6myc-tag fusion protein.

FORMULATION: 100 µg IgG in 100 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 reacts with Myc-tag on Immunocytochemistry and Flow cytometry.

APPLICATIONS:

Immunocytochemistry; 10 µg/mL
Flow cytometry; 5 µg/mL (final concentration)
*Please refer to the data sheet (MBL code no. M047-3) for other applications.

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

INTENDED USE:

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

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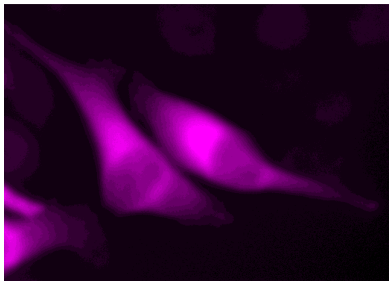
Clone PL14 is used in these references.



Immunocytochemical detection of Myc-Tag in transfectant with M047-A64.

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

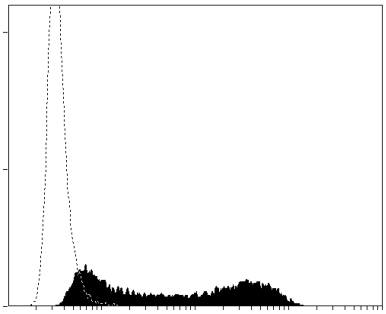
PROTOCOLS:

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (for example, spread $1x10^4$ 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 (PFA) for 10 minutes at room temperature.
4) Wash the glass slide 2 times with PBS.
5) Immerse the slide in PBS containing 0.1% Triton X-100 for 10 minutes at room temperature.
6) Wash the glass slide 2 times with PBS.
7) Add the primary antibody diluted with PBS 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) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
10) Promptly add mounting medium onto the slide, then put a cover slip on it.



Flow cytometric analysis of Myc-Tag expression in transfectant. *Open histogram indicates the reaction of isotypic control to the cells. Shaded histogram indicates the reaction of M047-A64 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_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 200 µL of Cytofix/Cytoperm™ solution (BD; code no. 554714) to the cell pellet after tapping. Mix well, then fix and permeabilize the cells for 20 minutes at 4°C.
3) Wash the cells 3 times with Perm/Wash™ buffer.
4) Add 20 µL of normal goat serum to the cell pellet after tapping. Mix well and incubate for 10 minutes at room temperature (20~25°C).
5) Add 20 µL of the primary antibody at the concentration as suggested in the **APPLICATIONS** diluted in the washing buffer. Mix well and incubate for 30 minutes at room temperature.
6) 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.
7) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

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