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

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Anti-HA-tag mAb-Alexa Fluor™ 647

CODE No.	M180-A64
CLONALITY	Monoclonal
CLONE	TANA2
ISOTYPE	Mouse IgG2b κ
QUANTITY	100 µL, 1 mg/mL
SOURCE	Purified IgG from hybridoma supernatant
IMMUNOGEN	KLH conjugated synthetic peptide, YPYDVPDYA (HA-tag)
REACTIVITY	This antibody reacts with N-terminal and C-terminal HA-tagged proteins.
FORMULATION	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.

APPLICATIONS-CONFIRMED

Immunocytochemistry	2-5 µg/mL
Flow cytometry	2-5 µg/mL

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

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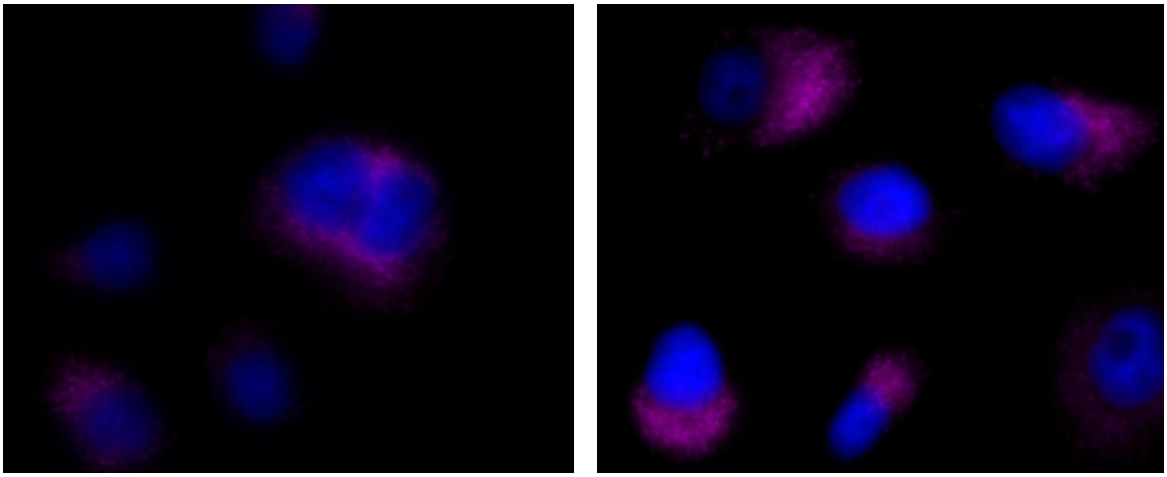
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

Immunocytochemistry

1) Spread the cells in the nutrient condition on a glass slide, then incubate in a CO_2 incubator for one night.
2) Remove the culture supernatant by careful aspiration.
3) Fix the cells by immersing the slide in 4% paraformaldehyde (PFA)/PBS for 10 min. at room temperature (20~25ºC).
4) Prepare a wash container such as a 500 mL beaker with a magnetic stirrer. Then wash the fixed cells on the glass slide by soaking the slide with a plenty of PBS in the wash container for 5 min. Take care not to touch the cells. Repeat another wash once more.
5) Immerse the slide in 0.2% Triton X-100/PBS for 10 min. at room temperature.
6) Wash the slide 2 times with PBS.
7) Cover each cell with normal goat serum for 5 min. at room temperature.
8) Add 200 μL of the primary antibody diluted with 2% fetal calf serum (FCS)/PBS as suggested in the **APPLICATIONS** onto the cells and incubate for 1 hr. at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
9) Wash the slide 2 times with PBS.
10) Counter stain with DAPI for 5 min. at room temperature.
11) Wash the slide 2 times with PBS.
12) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
13) Promptly add mounting medium onto the slide, then put a cover slip on it.



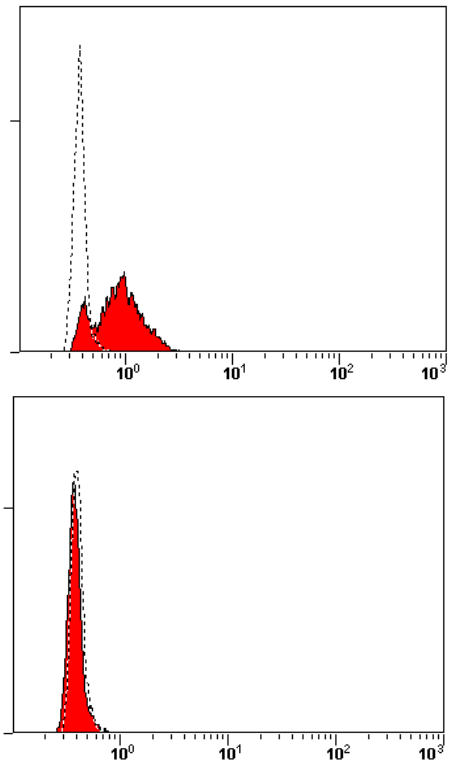
Immunocytochemical detection of HA-tagged protein in HeLa

Magenta: M180-A64
Blue: DAPI

Left: 2 μg/mL
Right: 5 μg/mL

Flow cytometric analysis for adherent cells

1) Detach the cells from culture dish.
2) Wash the cells 3 times with 1 mL of washing buffer [PBS containing 2% fetal calf serum (FCS)].
3) Add 200 µL of 4% paraformaldehyde (PFA) to the cell pellet after tapping. Mix well, then fix the cells for 10 min. at room temperature.
4) Wash the cells 2 times with 1 mL of washing buffer.
5) Add 200 µL of PBS containing 0.2% Triton X-100 to the cell pellet after tapping. Mix well, then permeabilize the cells for 10 min. at room temperature.
6) Wash the cells 1 time with 1 mL of washing buffer.
7) Resuspend the cells with washing buffer (4 x 10^6 cells/mL).
8) Add 100 µL of the cell suspension into each tube, and centrifuge at 500 x g for 1 min. at room temperature (20~25°C). Remove supernatant by careful aspiration.
9) Add 20 µL of Clear Back (human Fc receptor blocking reagent, MBL; code no. MTG-001) to the cell pellet after tapping. Mix well and incubate for 5 min. at room temperature.
10) Add 40 µL of the primary antibody at the concentration as suggested in the **APPLICATIONS** diluted in the washing buffer Mix well and incubate for 20 min. at room temperature.
11) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 min. at room temperature. Remove supernatant by careful aspiration. Repeat another wash once more.
12) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.



Flow cytometric detection of HA-tagged protein in HeLa

Closed: M180-A64 (2 µg/mL)
Open: Isotype control (M077-A64)

Upper: HA-tagged protein in HeLa
Lower: Parental cell (HeLa)