

# Anti-DDDDK-tag mAb-Alexa Fluor<sup>®</sup> 488

|                    |   |
|--------------------|---|
| <b>CODE No.</b>    | M185-A48  |
| <b>CLONALITY</b>   | Monoclonal  |
| <b>CLONE</b>       | FLA-1   |
| <b>ISOTYPE</b>     | Mouse IgG2a $\kappa$  |
| <b>QUANTITY</b>    | 100 $\mu$ L, 1 mg/mL  |
| <b>SOURCE</b>      | Purified IgG from hybridoma supernatant   |
| <b>IMMUNOGEN</b>   | KLH conjugated DYKDDDDK peptide   |
| <b>REACTIVITY</b>  | This antibody reacts with N-terminal, Internal and C-terminal DDDDK-tagged (DYKDDDDK) proteins. |
| <b>FORMULATION</b> | PBS containing 1% BSA and 0.1% ProClin 150.   |
| <b>STORAGE</b>     | This antibody solution is stable for one year from the date of purchase when stored at 4°C.     |

## APPLICATIONS-CONFIRMED

|                            |                  |
|----------------------------|------------------|
| <u>Immunocytochemistry</u> | 0.5-1 $\mu$ g/mL |
| <u>Flow cytometry</u>      | 0.5 $\mu$ g/mL   |

## REFERENCES

- 1) Kasubuchi, M., *et al.*, *Sci. Rep.* **7**, 5168 (2017) [IC]
- 2) Kobayashi, Y., *et al.*, *PLoS Pathog.* **12**, e1005785 (2016) [IC]

For more information, please visit our web site <https://ruo.mbl.co.jp/>.

## RELATED PRODUCTS

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

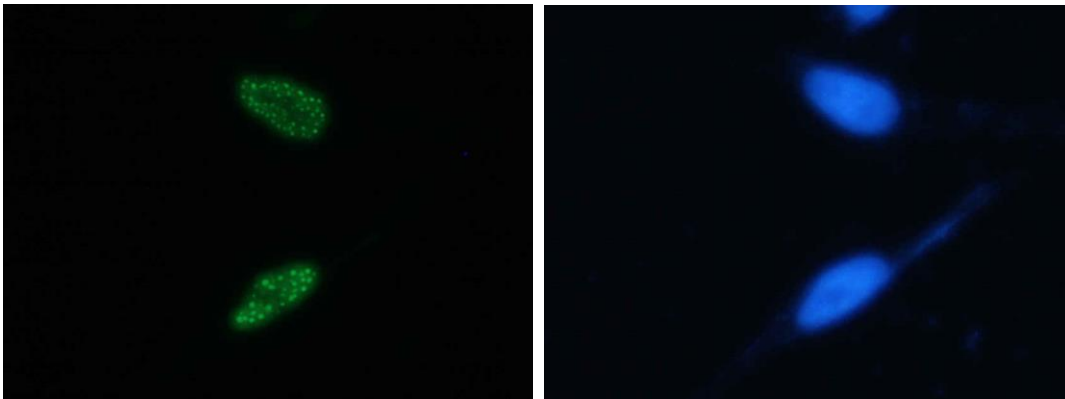
## LABEL LICENSES:

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### Immunocytochemistry

- 1) Spread the cells in the nutrient condition on a glass slide, then incubate in a CO<sub>2</sub> incubator overnight.
- 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 (20~25°C).
- 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 in a plenty of PBS as in the step 4).
- 7) Cover each cell with Clear Back (human Fc receptor blocking reagent, MBL, code no. MTG-001) for 5 min. at room temperature.
- 8) Add 200 µL of the primary antibody diluted with 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 in a plenty of PBS as in the step 4).
- 10) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
- 11) Counter stain with DAPI for 5 min. at room temperature.
- 12) Wash the slide in a plenty of PBS as in the step 4).
- 13) Promptly add mounting medium onto the slide, then put a cover slip on it.



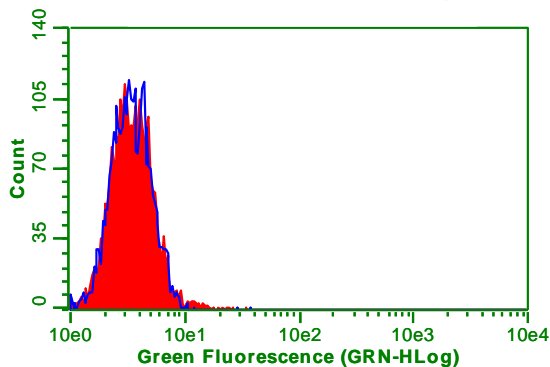
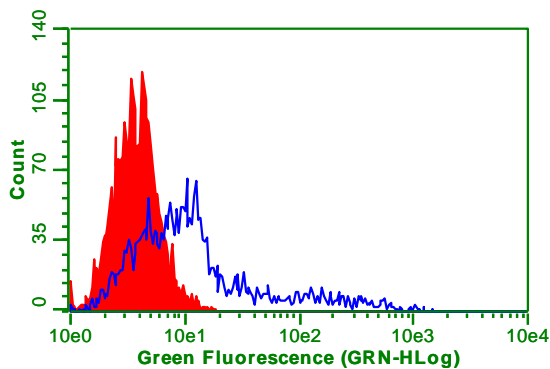
### ***Immunocytochemical detection of DDDDK-tagged protein in HeLa***

Green: M185-A48

Blue: DAPI

### Flow cytometric analysis for adherent cells

- 1) Detach the cells from culture dish.
- 2) Wash the cells once with 1 mL of washing buffer [PBS containing 2% fetal calf serum (FCS)].
- 3) Resuspend the cells with washing buffer ( $3 \times 10^6$  cells/mL).
- 4) Add 100  $\mu$ L 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.
- 5) Add 20  $\mu$ L 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.
- 6) Add 40  $\mu$ L of the primary antibody at the concentration as suggested in the **APPLICATIONS** diluted in the washing buffer. Mix well and incubate for 30 min. at room temperature.
- 7) 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.
- 8) Resuspend the cells with 500  $\mu$ L of the washing buffer and analyze by a flow cytometer.



### ***Flow cytometric detection of DDDDK-tagged protein in HeLa***

Open: M185-A48

Closed: Isotype control (MBL, code no. M076-A48)

Upper: DDDDK-tagged protein in HeLa

Lower: Parental cell (HeLa)