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MONOCLONAL ANTIBODY

Anti-RFP mAb

Code No.	Clone	Subclass	Quantity	Concentration
M155-3	8D6	Mouse IgG1 κ	100 µL	1 mg/mL

BACKGROUND: Expression vector containing a tag sequence is commonly used to introduce and express a specific gene into a target cell. Red Fluorescent Protein (RFP) fusion protein expression system is preferably used in various laboratories because of an easy monitoring of fusion proteins. This specific antibody for RFP is useful tool for monitoring of the fusion protein expression.

SOURCE: This antibody was purified from hybridoma (clone 8D6) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with C3H mouse lymphocyte immunized with RFP.

FORMULATION: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with RFP fusion proteins on Western blotting and Immunocytochemistry.

APPLICATIONS:

Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; Not tested*
*M165-3 and M165-8 are suitable for this application.
Immunohistochemistry; Not tested*
*It is reported that clone 8D6 can be used in this application in the reference number 1) and 5)-7).
Immunocytochemistry; 10 µg/mL
Flow cytometry; Not tested

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

REFERENCES:

1) Fujita, J., *et al.*, *Cell Rep*. **21**, 1562-1573 (2017) [IH]
2) Sato, Y., *et al.*, *Oncotarget* **8**, 39345-39355 (2017) [IC]
3) Matti, U., *et al.*, *Nat. Commun.* **4**, 1439 (2013) [WB]
4) Hihara, S., et al., *Cell Rep*. **2**, 1645-1656 (2012) [WB]
5) Morita, H., *et al.*, *Development* **139**, 1417-1426 (2012) [IH]
6) Chen, Q., *et al.*, *J. Cell Sci.* **125**, 2224-2234 (2012) [IH]
7) Bresciani, E., *et al., PLoS One* **5**, e14296 (2010) [IH]

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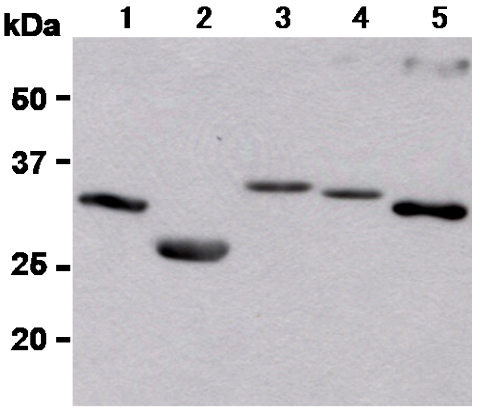


Western blot analysis of DsRed (1), mRFP1* (2), mCherry* (3), mOrange* (4) and mPlum* (5) using M155-3.
**Sample number (2) to (5) are provided by RIKEN.*

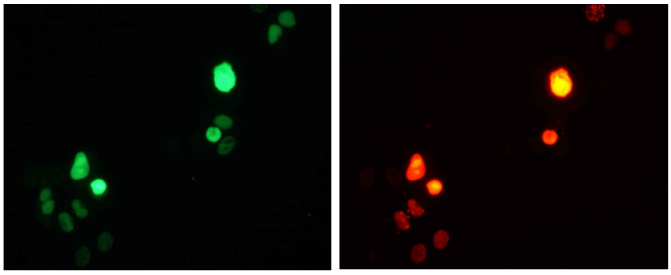
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

PROTOCOLS:

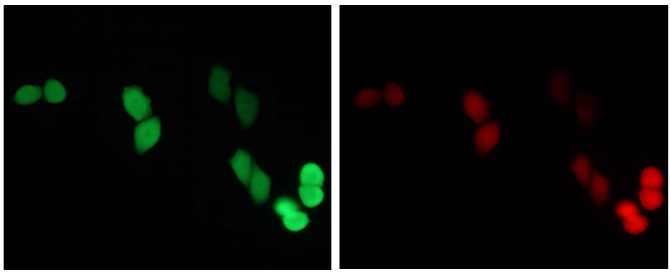
SDS-PAGE & Western Blotting

1) Mix the sample with equal volume of Laemmli's sample buffer.
2) Boil the samples for 3 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
4) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
5) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
6) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
7) Wash the membrane with PBS-T (5 minutes x 3 times).
8) Incubate the membrane with the 1:10,000 Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1%

skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
9) Wash the membrane with PBS-T (10 minutes x 3 times).
10) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
11) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
12) Expose to an X-ray film in a dark room for 3 minutes.
13) Develop the film as usual. The condition for exposure and development may vary.



Immunocytochemical detection of DsRed on 4% PFA fixed transfectant using M155-3 (left). Right panel is DsRed own fluorescence.



Immunocytochemical detection of mRFP1 on 4% PFA fixed transfectant* using M155-3 (left). Right panel is mRFP1 own fluorescence.

**This transfectant is provided by RIKEN.*

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) The glass slide was washed with PBS containing 2 % FCS 3 times.
5) Immerse the slide in PBS containing 0.2% Triton X-100 in PBS for 10 minutes at room temperature.
6) The glass slide was washed 3 times with PBS containing 2% FCS.
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) The glass slide was washed 3 times with PBS containing 2% FCS.
9) Add FITC-conjugated anti-mouse IgG antibody diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
10) The glass slide was washed 3 times with PBS containing 2% FCS.
11) Wipe excess liquid from 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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