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

MBL
A JSR Life Sciences Company

POLYCLONAL ANTIBODY

Anti-RFP pAb

Code No.	Quantity	Form
PM005	100 µL	Purified IgG

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 rabbit serum. The rabbit was immunized with RFP.

FORMULATION: 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. It reacts with DsRed, mRFP1, mCherry, mOrange, mPlum, tdTomato* and mStrawberry**.

It is reported in the reference number 2)*, 10)*, 12)**, 14)** and 16)**.

APPLICATIONS:

Western blotting; 1:1,000
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested*
*It is reported that this antibody can be used in this application in the reference number 1)-6), 9)-11) and 17).
Immunocytochemistry; 1:500
Flow cytometry; Not tested

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

INTENDED USE:

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

REFERENCES:

1) Shainer, I., *et al., Sci. Rep.* **7**, 44777 (2017) [IHC]
2) Woodard, L. E., *et al., Sci. Rep.* **7**, 4409 (2017) [IHC]
3) Fujii, M., *et al., Nat. Commun.* **8**, 14664 (2017) [IHC]
4) Tuschl, K., *et al., Nat. Commun.* **7**, 11601 (2016) [IHC]
5) Wang, N., *et al., Stem Cell Rep.* **6**, 668-678 (2016) [IHC]
6) Nagao, M., *et al., Nat. Commun.* **7**, 11102 (2016) [IHC]
7) Arimoto-Matsuzaki, K., *et al., Nat. Commun.* **7**, 10252 (2016) [IC]
8) Valbuena, N., *et al., J. Cell Sci.* **125**, 1920-1928 (2012) [WB]
9) Humphreys, B. D., *et al., Am. J. Pathol.* **176**, 85-97 (2010) [IHC]
10) Letzkus, J. J., *et al.*, *Nature* **480**, 331-335 (2011) [IHC]
11) Skora, A. D., and Spradling, A. C., *PNAS* **107**, 7389-7394 (2010) [IHC]
12) Ishida, Y., *et al., Mol. Biol. Cell* **20**, 2744-2754 (2009) [WB]
13) Bauer, P. O., *et al., J. Biol. Chem.* **284**, 13153-13164 (2009) [WB]
14) Fujita, N., *et al.*, *Mol. Biol. Cell* **19**, 4651-4659 (2008) [WB]
15) Wong, H. K., *et al.*, *Hum. Mol. Genet.* **17**, 3223-3235 (2008) [WB]
16) Fujita, N., *et al.*, *Mol. Biol. Cell* **19**, 2092-2100 (2008) [WB]
17) Matsuura, K., *et al., J. Cell Biol.* **167**, 351-363 (2004) [IHC]

As this product has been used in many researches, these references are a part of such reports.
Please visit our website at https://ruo.mbl.co.jp/.

RELATED PRODUCTS:

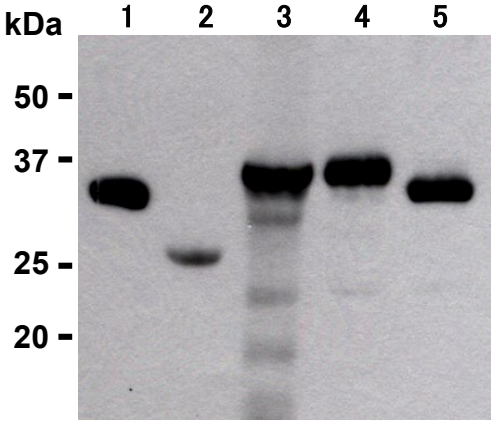
Please visit our website at https://ruo.mbl.co.jp/.

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

PROTOCOLS:

SDS-PAGE & Western blotting

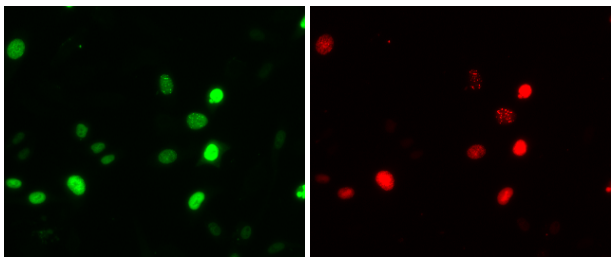
1) Mix the sample with equal volume of Laemmli's sample buffer.
2) Boil the samples for 3 minutes and centrifuge. Load 10 µL of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% methanol). See the manufacturer's manual for precise transfer procedure.
4) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
5) 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 the conditions.)
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3).
7) Incubate the membrane with the 1:10,000 anti-IgG (Rabbit) pAb-HRP (MBL, code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
8) Wash the membrane with PBS-T (10 minutes x 3).
9) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
10) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
11) Expose to an X-ray film in a dark room for 3 minutes.
12) Develop the film as usual. The condition for exposure and development may vary.



Western blotting analysis of DsRed (1), mRFP1* (2), mCherry* (3), mOrange* (4) and mPlum* (5) using PM005.
**Sample number (2) to (5) are provided by RIKEN.*

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (For example, spread the 1 x 10^4 cells for one slide, then incubate in a CO_2 incubator overnight.)
2) Wash the cells twice with PBS.
3) Fix the cells with PBS containing 4% paraformaldehyde for 10 minutes at room temperature.
4) Wash the cells twice with PBS.
5) Permeabilize the cells with 0.2% Triton X-100/PBS for 10 min. at room temperature.
6) Wash the slide twice with PBS.
7) Incubate the cells with the primary antibody diluted with PBS containing 2% fetal calf serum (FCS) as suggested in the **APPLICATIONS** for 1 hour at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
8) Wash the slide twice with PBS.
9) Incubate the cells with 1:500 Alexa Fluor® 488 Goat Anti-Rabbit IgG (Thermo Fisher Scientific, code no. A-11034) diluted with PBS containing 2% FCS for 30 minutes at room temperature. Keep out light by aluminum foil.
10) Wash the slide twice with PBS.
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.



Immunocytochemical detection of DsRed fusion protein expressed in HeLa transfectants.
Green: Anti-RFP pAb (PM005)
Red: DsRed fluorescence