

POLYCLONAL ANTIBODY

# Anti-Myc-tag pAb

Code No.	Quantity	Form
562	100 $\mu$ L	Rabbit IgG

**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 rabbit serum using protein A agarose. The rabbit was immunized with KLH conjugated synthetic peptide, KLH-EQKLISEEDL (Myc-tag).

**FORMULATION:** 100  $\mu$ L 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  $-20^{\circ}\text{C}$ .

**REACTIVITY:** This antibody reacts with Myc-tag on Western blotting.

## APPLICATIONS:

Western blotting; 1:1,000 for chemiluminescence detection system

Immunoprecipitation; 2  $\mu$ L

Chromatin immunoprecipitation (ChIP); Not tested\*

Immunohistochemistry; Not tested\*

Immunocytochemistry; 1:250

Flow cytometry; Not tested

ELISA; Not tested\*

\*It is reported that this antibody can be used in ChIP<sup>2)</sup>, Immunohistochemistry<sup>4), 5)</sup> and ELISA<sup>1)</sup>.

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

## INTENDED USE:

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

## REFERENCES:

- 1) Sangawa, T., *et al.*, *Protein Sci.* **22**, 840-850 (2013) [ELISA]
- 2) Isogai, E., *et al.*, *PLoS One* **6**, e19297 (2011) [ChIP]
- 3) Fukunaka, A., *et al.*, *J. Biol. Chem.* **286**, 16363-16373 (2011)
- 4) Shin, M., *et al.*, *Development* **136**, 595-603 (2009) [IHC]
- 5) Nakaya, Y., *et al.*, *Nat. Cell Biol.* **10**, 765-775 (2008) [IHC]
- 6) Nakamura, T., *et al.*, *Genes Dev.* **22**, 1244-1256 (2008)
- 7) Nadanaka, S., *et al.*, *Mol. Cell Biol.* **27**, 1027-1043 (2007)
- 8) Imamura, M., *et al.*, *Hum. Mol. Genet.* **14**, 775-783 (2005)
- 9) Harada, A., *et al.*, *J. Biol. Chem.* **280**, 18418-18424 (2005)
- 10) Oinuma, I., *et al.*, *J. Neurosci.* **24**, 11473-11480 (2004)
- 11) Naito, M., *et al.*, *Mol. Cell Biol.* **24**, 8418-8427 (2004)
- 12) Asada, M., *et al.*, *Mol. Cell Biol.* **24**, 8236-8243 (2004)
- 13) Onesto, C., *et al.*, *J. Biol. Chem.* **279**, 34217-34226 (2004)
- 14) Fujita, F., *et al.*, *Mol. Cell Biol.* **23**, 7780-7793 (2003)
- 15) Oinuma, I., *et al.*, *J. Biol. Chem.* **278**, 25671-25677 (2003)
- 16) Uchida, Y., *J. Biol. Chem.* **278**, 366-371 (2003)
- 17) Fujita, H., *et al.*, *J. Biol. Chem.* **277**, 45428-45434 (2002)
- 18) Vouret-Craviari, V., *et al.*, *J. Cell Sci.* **115**, 2475-2484 (2005)
- 19) Iwai, N., *et al.*, *Circulation* **105**, 41-47 (2002)
- 20) Kamimoto, T., *et al.*, *J. Biol. Chem.* **276**, 37520-37528 (2001)
- 21) Yasui, H., *et al.*, *J. Biol. Chem.* **276**, 15298-15305 (2001)
- 22) Inagaki, N., *et al.*, *J. Biol. Chem.* **275**, 27165-27171 (2000)
- 23) Yoshizawa, T., *et al.*, *Hum. Mol. Genet.* **9**, 69-78 (2000)
- 24) Kondo, T., *et al.*, *Mol. Cell Biol.* **19**, 1136-1143 (1999)
- 25) Itoh, M., *et al.*, *J. Biol. Chem.* **274**, 5981-5986 (1999)
- 26) Barral, Y., *et al.*, *Genes Dev.* **13**, 176-187 (1999)
- 27) Shimomura, T., *et al.*, *Mol. Cell Biol.* **18**, 5485-5491 (1998)

As this antibody is famous all over the world, a lot of researches have been reported. These references are a part of such reports.

kDa

97 -

66 -

45 -

30 -



**Western blot analysis of Myc-tag expression in TRAF6-Myc-tag transfected cells using 562.**

**PROTOCOLS:****SDS-PAGE & Western Blotting**

- 1) Mix the sample with equal volume of Laemmli's sample buffer.
- 2) Boil the samples for 2 minutes and centrifuge. Load 10  $\mu$ L 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<sup>2</sup> 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) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody to be used will depend on conditions.)
- 6) Wash the membrane with PBS (5 minutes x 6 times).
- 7) Incubate the membrane with 1:10,000 of 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 (5 minutes x 6 times).
- 9) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 10) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. The condition for exposure and development may vary.

**Immunoprecipitation**

- 1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer [50 mM Tris-HCl (pH 7.2), 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol] containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds)
- 2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube.
- 3) Add primary antibody as suggest in the **APPLICATIONS** into 200  $\mu$ L of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 20  $\mu$ L of 50% protein A agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
- 4) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
- 5) Resuspend the beads in 20  $\mu$ L of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10  $\mu$ L/lane for the SDS-PAGE analysis.  
(See **SDS-PAGE & Western blotting**.)

**Immunocytochemistry**

- 1) Culture the cells in the appropriate condition on a glass slide.

- 2) Fix the cells by immersing the slide in acetone for 10 minutes on ice.
- 3) Air dry the slides.
- 4) Add the primary antibody diluted with PBS as suggest 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.)
- 5) Prepare a wash container such as a 500 mL beaker with a stirrer. Then wash the cells on the glass slide by soaking the slide with a plenty of PBS in the wash container for 5 minutes. Take care not to touch the cells. Repeat another wash once more.
- 6) Add FITC conjugated anti-rabbit IgG antibody diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
- 7) Wash the slide in a plenty of PBS as in the step 5).
- 8) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
- 9) Promptly add mounting medium onto the slide, then put a cover slip on it.

**RELATED PRODUCTS****Antibodies**

M048-3	Anti-GFP mAb (1E4)
D153-3	Anti-GFP mAb (RQ2)
D153-6	Anti-GFP mAb-Biotin (RQ2)
D153-8	Anti-GFP mAb-Agarose (RQ2)
598	Anti-GFP pAb (polyclonal)
598-7	Anti-GFP pAb-HRP-Direct (polyclonal)
PM073	Anti-Renilla GFP pAb (polyclonal)
M208-3	Anti-RFP mAb Cocktail (1G9, 3G5)
M155-3	Anti-RFP mAb (8D6)
M165-3	Anti-RFP mAb (3G5)
M165-8	Anti-RFP mAb-Agarose (3G5)
M204-3	Anti-RFP mAb (1G9)
M204-7	Anti-RFP mAb-HRP-Direct (1G9)
PM005	Anti-RFP pAb (polyclonal)
PM005-7	Anti-RFP pAb-HRP-Direct (polyclonal)
M180-3	Anti-HA-tag mAb (TANA2) (200 $\mu$ L)
M180-6	Anti-HA-tag mAb-Biotin (TANA2)
M180-7	Anti-HA-tag mAb-HRP-Direct (TANA2)
561	Anti-HA-tag pAb (polyclonal) (0.1 mL)
561-7	Anti-HA-tag pAb-HRP-Direct (polyclonal)
561-8	Anti-HA-tag pAb-Agarose (polyclonal)
M132-3	Anti-HA-tag mAb (5D8)
M185-3L	Anti-DDDDK-tag mAb (FLA-1) (1 mL)
M185-7	Anti-DDDDK-tag mAb-HRP-Direct (FLA-1)
PM020	Anti-DDDDK-tag pAb (polyclonal)
PM020-7	Anti-DDDDK-tag pAb-HRP-Direct (polyclonal)
PM020-8	Anti-DDDDK-tag pAb-Agarose (polyclonal)
M192-3	Anti-Myc-tag mAb (My3) (200 $\mu$ L)
M192-6	Anti-Myc-tag mAb-Biotin (My3)
M047-3	Anti-Myc-tag mAb (PL14)
M047-6	Anti-Myc-tag mAb-Biotin (PL14)
M047-7	Anti-Myc-tag mAb-HRP-Direct (PL14)
M047-8	Anti-Myc-tag mAb-Agarose (PL14)
562	Anti-Myc-tag pAb (polyclonal) (0.1 mL)
D291-3	Anti-His-tag mAb (OGHis) (200 $\mu$ L)

D291-6	Anti-His-tag mAb-Biotin (OGHis)
D291-7	Anti-His-tag mAb-HRP-DirecT (OGHis)
D291-8	Anti-His-tag mAb-Agarose (OGHis)
D291-A48	Anti-His-tag mAb-Alexa Fluor® 488 (OGHis)
D291-A59	Anti-His-tag mAb-Alexa Fluor® 594 (OGHis)
D291-A64	Anti-His-tag mAb-Alexa Fluor® 647 (OGHis)
M089-3	Anti-His-tag mAb (6C4)
M136-3	Anti-His-tag mAb (2D8)
PM032	Anti-His-tag pAb (polyclonal)
PM032-8	Anti-His-tag pAb-Agarose (polyclonal)
M167-3	Anti-V5-tag mAb (1H6)
M215-3	Anti-V5-tag mAb (OZA3)
PM003	Anti-V5-tag pAb (polyclonal)
PM003-7	Anti-V5-tag pAb-HRP-DirecT (polyclonal)
PM003-8	Anti-V5-tag pAb-Agarose (polyclonal)
PM021	Anti-S-tag pAb (polyclonal)
PM070	Anti-E-tag pAb (polyclonal)
PM022	Anti-T7-tag pAb (polyclonal)
563	Anti-VSV-G-tag pAb (polyclonal)
M071-3	Anti-GST-tag mAb (3B2)
M209-3	Anti-GST-tag mAb (GT5)
PM022	Anti-GST-tag pAb (polyclonal)
M095-3	Anti-Luciferase mAb (2D4)
PM016	Anti-Luciferase pAb (polyclonal)
PM047	Anti-Renilla Luciferase pAb (polyclonal)
M094-3	Anti-β-galactosidase mAb (5A3)
PM049	Anti-β-galactosidase pAb (polyclonal)
M091-3	Anti-MBP (Maltose Binding Protein) mAb (1G12)
M013-3	Anti-Thioredoxin (Trx-tag) mAb (2C9)
PM015	Anti-CBD (Chitin Binding Domain) pAb (polyclonal)
PM071	Anti-Calmodulin Binding Protein-tag pAb (polyclonal)
M211-3	Anti-Strep-tag II mAb (4F1)
M214-3	Anti-mini-AID-tag mAb (1E4)

Smart-IP series

3190	Magnetic Rack
M180-11	Anti-HA-tag mAb-Magnetic Beads (TANA2)
M132-11	Anti-HA-tag mAb-Magnetic Beads (5D8)
M185-11	Anti-DDDDK-tag mAb-Magnetic Beads (FLA-1)
M047-11	Anti-Myc-tag mAb-Magnetic Beads (PL14)
D291-11	Anti-His-tag mAb-Magnetic Beads (OGHis)
D153-11	Anti-GFP mAb-Magnetic Beads (RQ2)
M165-11	Anti-RFP mAb-Magnetic Beads (3G5)
M198-9	Anti-E-tag mAb-Magnetic beads (21D11)
M167-11	Anti-V5-tag mAb-Magnetic Beads (1H6)
D058-9	Anti-Multi Ubiquitin mAb-Magnetic beads (FK2)
M075-11	Mouse IgG1 (isotype control)-Magnetic Beads
M076-11	Mouse IgG2a (isotype control)-Magnetic Beads
M077-11	Mouse IgG2b (isotype control)-Magnetic Beads
M081-11	Rat IgG2a (isotype control)-Magnetic Beads
M180-10	Anti-HA-tag mAb-Magnetic Agarose (TANA2)
M132-10	Anti-HA-tag mAb-Magnetic Agarose (5D8)
M185-10	Anti-DDDDK-tag mAb-Magnetic Agarose (FLA-1)
M047-10	Anti-Myc-tag mAb-Magnetic Agarose (PL14)
D291-10	Anti-His-tag mAb-Magnetic Agarose (OGHis)
D153-10	Anti-GFP mAb-Magnetic Agarose (RQ2)
M165-10	Anti-RFP mAb-Magnetic Agarose (3G5)
M167-10	Anti-V5-tag mAb-Magnetic Agarose (1H6)
M198-10	Anti-E-tag mAb-Magnetic Agarose (21D11)

Protein Purification Kits

3320	HA-tagged Protein PURIFICATION KIT
3342	HA-tagged Protein Magnetic PURIFICATION KIT
3321	HA-tagged Protein PURIFICATION GEL (1 mL)
3320-205	HA-tag peptide (2 mg x 5)
3325	DDDDK-tagged Protein PURIFICATION KIT
3343	DDDDK-tagged Magnetic Protein PURIFICATION KIT
3326	DDDDK-tagged Protein PURIFICATION GEL with Elution Peptide (1 mL gel, 5 mg peptide)
3326K	DDDDK-tagged Protein PURIFICATION CARTRIDGE
3328	DDDDK-tagged Protein PURIFICATION GEL (5 mL gel)
3325-205	DDDDK-tag peptide (1 mg x 5)
3305	c-Myc-tagged Protein MILD PURIFICATION KIT Ver. 2
3340	c-Myc-tagged Protein Magnetic PURIFICATION KIT
3306K	c-Myc-tagged Protein PURIFICATION CARTRIDGE
3306	c-Myc-tagged Protein MILD PURIFICATION GEL (1 mL gel, 1 mg peptide)
3300-205	c-Myc tag peptide (EQKLISEEDL) (1 mg x 5)
3310	His-tagged Protein PURIFICATION KIT
3311	His-tagged Protein PURIFICATION GEL (1 mL gel, 5 mg peptide)
3315	V5-tagged Protein PURIFICATION KIT
3341	V5-tagged Magnetic Protein PURIFICATION KIT
3316	V5-tagged Protein PURIFICATION GEL (1 mL)
3315-205	V5-tag peptide (2 mg x 5)

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