

POLYCLONAL ANTIBODY

# Anti-Myc-tag

Code No.  
562-5

Quantity  
500 µL

Form  
Rabbit IgG

**BACKGROUND:** Epitope tagging has widely been accepted technique that fuse 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:** 500 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 Myc-tag on Western blotting.

**APPLICATIONS:**

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

Immunoprecipitation: 2 µL

Immunohistochemistry: Not tested

Immunocytochemistry: 1:250

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:**

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- 22) Itoh, M., *et al.*, *J. Biol. Chem.* **274**, 5981-5986 (1999)
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- 24) Shimomura, T., *et al.*, *Mol. Cell Biol.* **18**, 5485-5491 (1998)

This antibody is used in these references.

kDa

97 -  
66 -  
45 -  
30 -



**Western blot analysis of Myc-tag expression in TRAF6-Myc-tag transfected cells using 562-5.**

**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 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<sup>2</sup> for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacturer'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 be depend on condition.)
- 6) Wash the membrane with PBS (5 minutes x 6 times).
- 7) Incubate the membrane with the 1:10,000 HRP-conjugated anti-rabbit IgG (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 µL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4 °C. Add 20 µL 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 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10 µL/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 are 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 washes once more.
- 6) Add 30 µL of 1:40 FITC conjugated anti-rabbit IgG (MBL; code no. IM-0833) 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**

562	anti-Myc-tag (polyclonal) (0.1 mL)
562-5	anti-Myc-tag (polyclonal) (0.5 mL)
M047-3	anti-Myc-tag (PL14)
M047-6	anti-Myc-tag-biotin (PL14)
M047-7	anti-Myc-tag HRP-Direct (PL14)
M047-8	anti-Myc-tag-agarose (PL14)
M047-A48	anti-Myc-tag-Alexa Fluor <sup>®</sup> 488 (PL14)
M047-A59	anti-Myc-tag-Alexa Fluor <sup>®</sup> 594 (PL14)
M047-A64	anti-Myc-tag-Alexa Fluor <sup>®</sup> 647 (PL14)
561	anti-HA-tag (polyclonal) (0.1 mL)
561-5	anti-HA-tag (polyclonal) (0.5 mL)
561-7	anti-HA-tag HRP-Direct (polyclonal)
561-8	anti-HA-tag-agarose (polyclonal)
M180-3	anti-HA-tag (TANA2) (200 µL)
M180-3S	anti-HA-tag (TANA2) (50 µL)
M180-A48	anti-HA-tag-Alexa Fluor <sup>®</sup> 488 (TANA2)
M180-A59	anti-HA-tag-Alexa Fluor <sup>®</sup> 594 (TANA2)
M180-A64	anti-HA-tag-Alexa Fluor <sup>®</sup> 647 (TANA2)
M132-3	anti-HA-tag (5D8)
M185-3L	anti-DDDDK-tag (FLA-1) (1 mL)
M185-3LL	anti-DDDDK-tag (FLA-1) (5 mL)
M185-3S	anti-DDDDK-tag (FLA-1) (50 µL)
M185-A48	anti-DDDDK-tag-Alexa Fluor <sup>®</sup> 488 (FLA-1)
M185-A59	anti-DDDDK-tag-Alexa Fluor <sup>®</sup> 594 (FLA-1)
M185-A64	anti-DDDDK-tag-Alexa Fluor <sup>®</sup> 647 (FLA-1)
PM020	anti-DDDDK-tag (polyclonal)
PM020-7	anti-DDDDK-tag HRP-Direct (polyclonal)
PM020-8	anti-DDDDK-tag-agarose (polyclonal)
D291-3	anti-His-tag (OGHis) (200 µL)
D291-3S	anti-His-tag (OGHis) (50 µL)
D291-6	anti-His-tag-biotin (OGHis)
D291-7	anti-His-tag HRP-Direct (OGHis)
D291-8	anti-His-tag-agarose (OGHis)
D291-A48	anti-His-tag-Alexa Fluor <sup>®</sup> 488 (OGHis)
D291-A59	anti-His-tag-Alexa Fluor <sup>®</sup> 594 (OGHis)
D291-A64	anti-His-tag-Alexa Fluor <sup>®</sup> 647 (OGHis)
M089-3	anti-His-tag (6C4)
M136-3	anti-His-tag (2D8)
PM032	anti-His-tag (polyclonal)

PM032-8	anti-His-tag-agarose (polyclonal)	3312	His-tagged Protein PURIFICATION GEL (5 mL gel, 50 mg peptide)
598	anti-GFP (polyclonal)	3315	V5-tagged Protein PURIFICATION KIT
598-7	anti-GFP HRP-Direct (polyclonal)	3315A	V5-tagged Protein PURIFICATION KIT (Trial Kit)
M048-3	anti-GFP (1E4)	3320	HA-tagged Protein PURIFICATION KIT
D153-3	anti-GFP (RQ2)	3320A	HA-tagged Protein PURIFICATION KIT (Trial Kit)
D153-A48	anti-GFP-Alexa Fluor <sup>®</sup> 488 (RQ2)	3325	DDDDK-tagged Protein PURIFICATION KIT
D153-A59	anti-GFP-Alexa Fluor <sup>®</sup> 594 (RQ2)	3325A	DDDDK-tagged Protein PURIFICATION KIT (Trial Kit)
D153-A64	anti-GFP-Alexa Fluor <sup>®</sup> 647 (RQ2)	3325-205	DDDDK-tag peptide (5 mg)
D153-8	anti-GFP-agarose (RQ2)	3326	DDDDK-tagged Protein PURIFICATION GEL with Elution Peptide (1 mL gel, 5 mg peptide)
PM005	anti-RFP (polyclonal)	3327	DDDDK-tagged Protein PURIFICATION GEL with Elution Peptide (5 mL gel, 25 mg peptide)
PM005-7	anti-RFP HRP-Direct (polyclonal)	3328	DDDDK-tagged Protein PURIFICATION GEL (5 mL gel)
M155-3	anti-RFP (8D6)	3329	DDDDK-tagged Protein PURIFICATION GEL (25 mL gel)
M165-3	anti-RFP (3G5)		
M165-8	anti-RFP-agarose (3G5)		
M071-3	anti-GST-tag (3B2)		
PM013	anti-GST-tag (polyclonal)		
PM013-7	anti-GST-tag HRP-Direct (polyclonal)		
M013-3	anti-Thioredoxin (2C9)		
M094-3	anti- $\beta$ -galactosidase (5A3)		
PM049	anti- $\beta$ -galactosidase (polyclonal)		
M095-3	anti-Luciferase (2D4)		
PM016	anti-Luciferase (polyclonal)		
PM047	anti-Renilla Luciferase (polyclonal)		
M091-3	anti-MBP (1G12)		
PM015	anti-Chitin Binding Domain (polyclonal)		
PM021	anti-S-tag (polyclonal)		
PM021-8	anti-S-tag-agarose (polyclonal)		
PM022	anti-T7-tag (polyclonal)		
PM022-8	anti-T7-tag-agarose (polyclonal)		
M167-3	anti-V5-tag (1H6)		
PM003	anti-V5-tag (polyclonal)		
PM003-7	anti-V5-tag HRP-Direct (polyclonal)		
PM003-8	anti-V5-tag-agarose (polyclonal)		
563	anti-VSV-G-tag (polyclonal)		
563-8	anti-VSV-G-tag-agarose (polyclonal)		

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

#### Smart-IP series

3190	Magnetic Rack
M047-9	anti-Myc-tag-magnetic beads (PL14)
M132-9	anti-HA-tag-magnetic beads (5D8)
M185-9	anti-DDDDK-tag-magnetic beads (FLA-1)
D291-3	anti-His-tag-magnetic beads (OGHis)
D153-9	anti-GFP-magnetic beads (RQ2)
M165-9	anti-RFP-magnetic beads (3G5)
M167-9	anti-V5-tag-magnetic beads (1H6)

#### Protein Purification Kit

3305	c-Myc-tagged Protein MILD PURIFICATION KIT
3305A	c-Myc-tagged Protein MILD PURIFICATION KIT (Trial Kit)
3306	c-Myc-tagged Protein MILD PURIFICATION GEL (1 mL gel, 1 mg peptide)
3307	c-Myc-tagged Protein MILD PURIFICATION GEL (5 mL gel, 5 mg peptide)
3300-205	c-Myc tag peptide (5 mg)
3310	His-tagged Protein PURIFICATION KIT
3310A	His-tagged Protein PURIFICATION KIT (Trial Kit)
3310-205	His-tag peptide (10mg)
3311	His-tagged Protein PURIFICATION GEL (1 mL gel, 10 mg peptide)