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

MBL

POLYCLONAL ANTIBODY

Anti-VSV-G-tag

Code No.	Quantity	Form
563	100 µL	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 ammonium sulfate precipitation. The rabbit was immunized with KLH-YTDIEMNRLGK.

FORMULATION: 100 µL volume of PBS containing 0.09% NaN_3.
*Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at 4°C.

REACTIVITY: This antibody reacts with VSV-G-tagged fusion proteins on Western blotting and Immunocytochemistry.

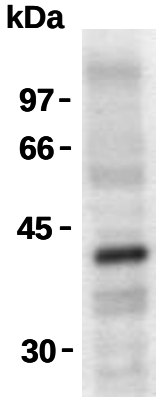
APPLICATIONS:
Western blotting; 1:100 for chemiluminescence detection system
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunocytochemistry; 1:100
Flow cytometry; Not tested

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

INTENDED USE:
For Research Use Only. Not for use in diagnostic procedures.

REFERENCES:
1) Patterson, A. M., *et al.*, *Inflamm. Bowel Dis.* **18**, 1112-1126 (2012)
2) Page, A., *et al.*, *J. Invest. Dermatol.* **130**, 1598-1610 (2010)
3) Smetsers, T. F., *et al.*, *Cancer Res.* **63**, 2965-2970 (2003)
4) Fanning, A. S., *et al.*, *FASEB J.* **16**, 1835-1837 (2002)
5) Nix, S. L., *et al.*, *J. Biol. Chem.* **275**, 41192-41200 (2000)
6) Okada, Y., *et al.*, *J. Biol. Chem.* **275**, 17016-17023 (2000)

This antibody is used in these references.



Western blot analysis of VSV-G-Tag expression in transfectant using 563.

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 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 will depend on the conditions.)
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 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-T (10 minutes x 3 times).
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.

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (for example, spread the $1x10^4$ 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 for 10 minutes at room temperature.
4) The glass slide was washed 3 times with PBS.
5) Immerse the slide in PBS containing 0.1% TritonX-100 for 10 minutes at room temperature.
6) The glass slide was washed 3 times with PBS.
7) 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.)
8) The glass slide was washed with PBS 3 times.
9) Add 100 µL of 1:100 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.
10) The glass slide was washed with PBS 3 times.
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.

RELATED PRODUCTS:

Antibodies

563	anti-VSV-G-tag (polyclonal)
563-8	anti-VSV-G-tag-agarose (polyclonal)
M185-3L	anti-DDDDK-tag (FLA-1)
PM020	anti-DDDDK-tag (polyclonal)
M180-3	anti-HA-tag (TANA2)
561	anti-HA-tag (polyclonal)
M132-3	anti-HA-tag (5D8)
D291-3	anti-His-tag (OGHis)
M089-3	anti-His-tag (6C4)
M136-3	anti-His-tag (2D8)
PM032	anti-His-tag (polyclonal)
598	anti-GFP (polyclonal)
M048-3	anti-GFP (1E4)
D153-3	anti-GFP (RQ2)
PM005	anti-RFP (polyclonal)
M155-3	anti-RFP (8D6)
M165-3	anti-RFP (3G5)
M192-3	anti-Myc-tag (My3)
562	anti-Myc-tag (polyclonal)
PM021	anti-S-tag (polyclonal)
PM022	anti-T7-tag (polyclonal)
M167-3	anti-V5-tag (1H6)
PM003	anti-V5-tag (polyclonal)
M071-3	anti-GST-tag (3B2)
PM013	anti-GST-tag (polyclonal)

Smart-IP series

3190	Magnetic Rack
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)
M132-9	anti-HA-tag-magnetic beads (5D8)
M180-9	anti-HA-tag-Magnetic beads (TANA2)
M047-9	anti-Myc-tag-magnetic beads (PL14)
M167-9	anti-V5-tag-magnetic beads (1H6)

Protein Purification Kit

3305	c-Myc-tagged Protein Mild Purification Kit
3306	c-Myc-tagged Protein Mild Purification Gel with Elution Peptide (1 mL gel, 1 mg peptide)
3300-205	c-Myc-tag peptide (5 mg)
3310	His-tagged Protein Purification Kit
3310-205	His-tag peptide (10mg)
3311	His-tagged Protein Purification Gel with Elution Peptide (1 mL gel, 10 mg peptide)
3315	V5-tagged Protein Purification Kit
3320	HA-tagged Protein Purification Kit
3321	HA-tagged Protein Purification Gel (1 mL)
3320-205	HA-tag peptide (10 mg)
3325	DDDDK-tagged Protein Purification Kit
3326	DDDDK-tagged Protein Purification Gel with Elution Peptide (1 mL gel, 5 mg peptide)
3328	DDDDK-tagged Protein Purification Gel (5 mL gel)
3325-205	DDDDK-tag peptide (5 mg)

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