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POLYCLONAL ANTIBODY

Anti-VSV-G-tag pAb

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

FORMULATION: 100 µL volume of PBS containing 50% glycerol, pH 7.2.

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

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

APPLICATIONS:
Western blotting; 1:100
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested*
*It is reported that this antibody can be used in this application in the reference number 1) and 3).
Immunocytochemistry; 1:100
Flow cytometry; Not tested

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

INTENDED USE:
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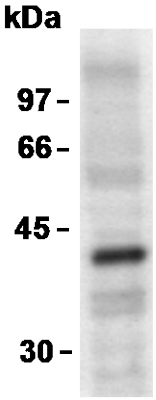
REFERENCES:
1) Nagamine, S., *et al., J. Biol. Chem.* **287**, 9579-9590 (2012) [IHC]
2) Patterson, A. M., *et al., Inflamm. Bowel Dis.* **18**, 1112-1126 (2012)
3) Page, A., *et al., J. Invest. Dermatol.* **130**, 1598-1610 (2010) [IHC]
4) Nix, S. L., *et al., J. Biol. Chem.* **275**, 41192-41200 (2000) [WB]
5) Van Itallie, C. M. and Anderson, J. M., *J. Cell Sci.* **110**, 1113-1121 (1997) [IC]
6) Meyerhardt, J. A., *et al., Oncogene* **14**, 1129-1136 (1997) [WB]



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

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 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 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-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) Wash the glass slide 3 times with PBS.
5) Immerse the slide in PBS containing 0.1% TritonX-100 for 10 minutes at room temperature.
6) Wash the glass slide 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) Wash the glass slide 3 times with PBS.
9) Add 100 µL of 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.
10) Wash the glass slide 3 times 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.

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