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

MONOCLONAL ANTIBODY

Anti-Phosphorylated Vimentin (Ser71) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D093-3	TM71	Rat IgG2a	100 µL	1 mg/mL

BACKGROUND: Components of intermediate filaments provide information on the origin of vertebrate cells. Antibody to vimentin can be used as to identify the vimentin subclass of intermediate filaments. Vimentin is a ~58 kDa, widely expressed protein that thought to be involved mainly in structural processes, such as wound healing. Scientists have also recently determined that activated human macrophages secrete vimentin into the extracellular space, and overproduction of vimentin has been associated with cellular senescence.

SOURCE: This antibody was purified from rat ascites fluid using protein G agarose. This hybridoma (clone TM71) was established by fusion of mouse myeloma cell SP2/0-Ag14 with Jcl:Wistar rat's medial iliac lymph node immunized with the KLH conjugated phospho-peptide PV71 (CAVLRpSSVPGV).

FORMULATION: 100 µg IgG in 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 recognizes the site-specific phosphorylation of vimentin at Ser71 on Western blotting and Immunocytochemistry.

APPLICATIONS:

Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunocytochemistry; 1-5 µg/mL
Flow cytometry; Not tested

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	U251	NIH/3T3	3Y1-B
Reactivity on WB	+	+	+

INTENDED USE:

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REFERENCES:

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3) Oguri, T., *et al., Genes Cells* **11**, 531-540 (2006)
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6) Nakamura, Y., *et al., Genes Cells* **5**, 823-837 (2000)
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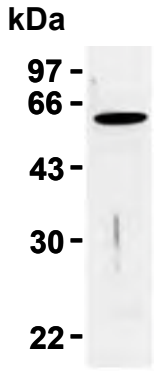
11) Kosako, H., *et al., Oncogene* **18**, 2783-88 (1999)
12) Ando, S., *et al., J. Biochem.* **122**, 409-14 (1997)

Clone TM71 is used in reference number 1)-10).



Western blot analysis of phosphorylated vimentin (Ser71) expression in U251 using D093-3.

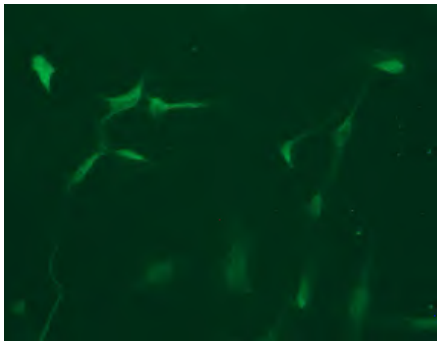
PROTOCOLS:

SDS-PAGE & Western Blotting

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. Measure the protein concentration of the supernatant and add the cold Lysis buffer to make 8 mg/mL solution.

3) Mix the sample with equal volume of Laemmli's sample buffer.
4) Boil the samples for 3 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
5) 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 manufacture's manual for precise transfer procedure.
6) 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.
7) 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 condition.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
9) Incubate the membrane with the 1:5,000 Anti-IgG (Rat) pAb-HRP (MBL; code no. IM-0825) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS-T (10 minutes x 3 times).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
12) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
13) Expose to an X-ray film in a dark room for 3 minutes.
14) Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; U251, NIH/3T3, 3Y1-B)



Immunocytochemical detection of phosphorylated vimentin (Ser71) on 4% PFA fixed U251 cells with D093-3.

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (for example, spread 1x10^4 of 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 (PFA) for 20 minutes at room temperature.
4) The glass slide was washed with PBS 3 times.
5) Immerse the slide in PBS containing 0.1% Triton X-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 3 times with PBS.
9) Add 100 µL of 1:100 Anti-IgG (Rat) pAb-FITC (MBL; code no. 354) 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 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.

(Positive control for Immunocytochemistry; U251)

RELATED PRODUCTS:

D096-3S	Anti-Phosphorylated Vimentin (Ser6) mAb (MO6)
D099-3S	Anti-Phosphorylated Vimentin (Ser33) mAb (YT33)
D094-3S	Anti-Phosphorylated Vimentin (Ser38) mAb (TM38)
D093-3S	Anti-Phosphorylated Vimentin (Ser71) mAb (TM71)
D122-3S	Anti-Phosphorylated Vimentin (Ser50) mAb (TM50)
D076-3S	Anti-Phosphorylated Vimentin (Ser55) mAb (4A4)
D095-3S	Anti-Phosphorylated Vimentin (Ser82) mAb (MO82)
PD005	Anti-Vimentin Fragment (V1) pAb (polyclonal)
D097-3S	Anti-GFAP (Glial Fibrillary Acidic Protein) mAb (MO389)
D098-3S	Anti-Phosphorylated GFAP (Thr7) mAb (TMG7)