

MONOCLONAL ANTIBODY

Anti-Phosphorylated Vimentin (Ser55) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D076-3	4A4	Mouse IgG2b	100 µL	1 mg/mL

BACKGROUND: Vimentin is an intermediate filament protein distributed widely in the cytoplasm and is phosphorylated by several protein kinases *in vitro*. Ser⁵⁵ residues on vimentin were reported to be one of the phosphorylation sites of vimentin at metaphase and were the phosphorylation sites for cdc2 kinase but not for cAMP-dependent protein kinase, protein kinase C, and Ca²⁺-calmodulin-dependent protein kinase II *in vitro*. Immunofluorescence and immunoelectron microscopy showed that vimentin Ser⁵⁵ residues distributed in the entire cytoplasmic vimentin filament system are phosphorylated when the cells enter mitosis and de-phosphorylated in cytokinesis. The use of this antibody that specifically reacts with the phosphorylation site of vimentin Ser⁵⁵ by cdc2 kinase enables estimation of a particular cdc2 kinase function.

SOURCE: This antibody was purified from hybridoma (clone 4A4) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell SP2/0-Ag14 with Balb/c mouse splenocyte immunized with the synthetic MPV55 phosphopeptide corresponding to mouse phosphorylated vimentin Ser⁵⁵ (SLYSS-phosphoS⁵⁵-PGGAYC-KLH).

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 reacts specifically with the phosphorylated MPV55 peptide but not the non-phosphorylated peptide. This antibody detects vimentin phosphorylated by cdc2 kinase and does not detect non-phosphorylated vimentin or phosphorylated vimentin by cAMP-dependent kinase, protein kinase C, or Ca²⁺-calmodulin-dependent protein kinase II on Western blotting.

SPECIES CROSS REACTIVITY:

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

APPLICATIONS:

Western blotting; 1-5 µg/mL

Immunoprecipitation; Not tested

Immunohistochemistry; Not tested*

*It is reported that this antibody can be used in Immunohistochemistry in the reference number 1)-10), 14) and 15).

Immunocytochemistry; 1 µg/mL

Flow cytometry; Not tested

ELISA; Not tested*

*It is reported that this antibody can be used in ELISA in the reference number 16).

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

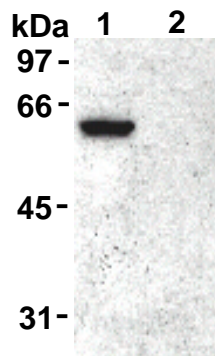
INTENDED USE:

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

REFERENCES:

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- 10) Minobe, S., *et al.*, *Neurosci. Res.* **63**, 294-301 (2009) [IHC]
- 11) Tatsumi, Y., *et al.*, *J. Cell Sci.* **119**, 3128-3140 (2006) [WB]
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- 13) Tsai, J. W., *et al.*, *J. Cell Biol.* **170**, 935-945 (2005) [IC]
- 14) Englund, C., *et al.*, *J. Neurosci.* **25**, 247-251 (2005) [IHC]
- 15) Miyata, T., *et al.*, *Development* **131**, 3133-3145 (2004) [IHC]
- 16) Tsujimura, K., *et al.*, *J. Biol. Chem.* **269**, 31097-31106 (1994) [WB, IC, ELISA]

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Western blotting analysis of phosphorylated Vimentin (Ser⁵⁵) in U251 cells, M phase (1) and interphase (2) using D076-3.

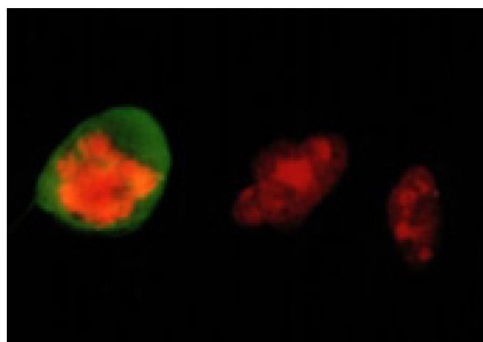
PROTOCOLS:

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 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 2 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
- 5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm² for 1 hour in a semi-dry transfer system. (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% methanol). 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 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
- 8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (10 minutes x 3).
- 9) Incubate the membrane with 1:10,000 Anti-IgG(H+L chain) (Mouse) pAb-HRP (MBL, code no. 330) 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).
- 11) 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.

- 12) Expose to an X-ray film in a dark room for 1 minute. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; U251)



Immunocytochemical detection of phosphorylated Vimentin (Ser⁵⁵) on formaldehyde fixed U251 cells with D076-3.
Green: D076-3
Red: Propidium iodide (PI)

Immunocytochemistry

- 1) Culture the cells in the appropriate condition on a glass slide. (For example, spread 1 x 10⁴ cells of U251 cells for one slide, then incubate in a CO₂ incubator overnight.)
- 2) Wash the cells 3 times with PBS.
- 3) Fix the cells by immersing the slide in PBS containing 3.7% formaldehyde for 10 minutes at room temperature.
- 4) The glass slide was washed with PBS 3 times. Immerse the slide in PBS containing 0.1% TritonX-100 for 10 minutes at room temperature.
- 5) The glass slide was washed 3 times with PBS.
- 6) Add the primary antibody diluted with PBS as suggested in the **APPLICATIONS** onto the cells and incubate for 1 hour at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
- 7) The glass slide was washed 3 times with PBS.
- 8) Add FITC-conjugated anti-mouse IgG antibody diluted with PBS onto the cells. Incubate for 1 hour at room temperature. Keep out light by aluminum foil.
- 9) The glass slide was washed 3 times with PBS.
- 10) Incubate the cells with 1 µg/mL of propidium iodide (PI) for 15 minutes at room temperature.
- 11) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
- 12) Promptly add Lab Vision™ PermaFluor™ Aqueous Mounting Medium (Thermo Fisher Scientific, code no. TA-006-FM) onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; U251)