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Not for use in diagnostic procedures.

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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*. Ser^{55} 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 Ca^{2+}-calmodulin-dependent protein kinase II *in vitro*. Immunofluorescence and immunoelectron microscopy showed that vimentin Ser^{55} 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 Ser^{55} 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 Ser^{55} (SLYSS-phosphoS^{55}-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 Ca^{2+}-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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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]
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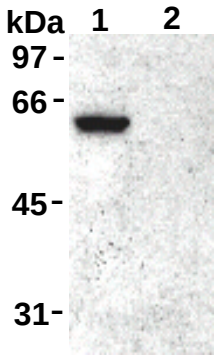
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.



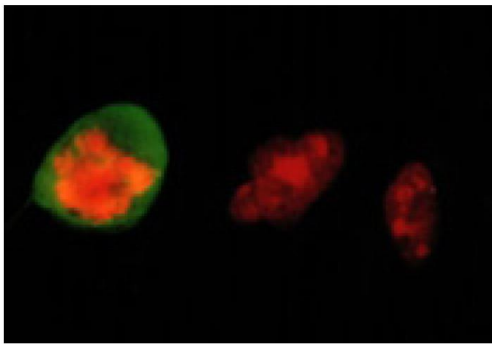
Western blotting analysis of phosphorylated Vimentin (Ser[55]) in U251 cells, M phase (1) and interphase (2) using D076-3.

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/cm^2 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 (Ser[55]) 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 10^4 cells of U251 cells for one slide, then incubate in a CO_2 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)