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Product datasheet for AM26448AF-N

Vimentin (VIM) pSer55 Mouse Monoclonal Antibody [Clone ID: 4A4]

Product data:

Product Type:	Primary Antibodies
Clone Name:	4A4
Applications:	IF, WB
Recommended Dilution:	Western blot: 1-5 µg/mL for chemiluminescence detection system. Immunocytochemistry: 1 µg/ml. For details see protocols below.
Reactivity:	Human, Mouse, Rat
Host:	Mouse
Isotype:	IgG2b
Clonality:	Monoclonal
Immunogen:	Synthetic MPV55 phosphopeptide corresponding to mouse phosphorylated vimentin Ser55.
Specificity:	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.
Formulation:	PBS containing 50% glycerol, pH 7.2. No preservative is contained. State: Azide Free State: Liquid Ig fraction
Concentration:	lot specific
Purification:	Protein A agarose
Conjugation:	Unconjugated
Storage:	Store (in aliquots) at -20 °C. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Gene Name:	vimentin
Database Link:	Entrez Gene 7431 Human P08670

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Background: Vimentin is an intermediate filament protein distributed widely in the cytoplasm and is phosphorylated by several protein kinase 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.

Synonyms: VIM

Note: This product was originally produced by MBL International.

Protocol:

SDS-PAGE & Western Blotting

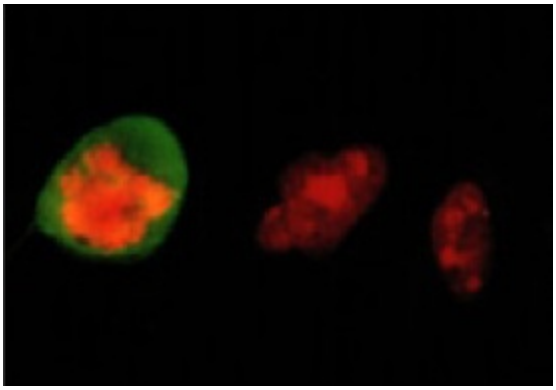
1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4oC with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4oC 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% 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 4oC.
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] (10 minutes x 3 times).
9) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG 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. 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)

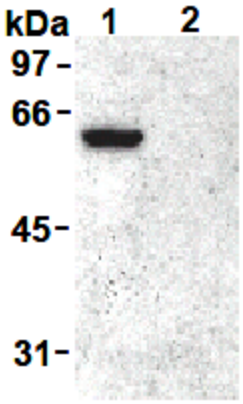
Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (For example, spread 1x10e4 cells of U251 cells for one slide, then incubate in a CO2 incubator for one night.)
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.
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 1 hour at room temperature. (Optimization of antibody concentration or incubation condition are recommended if necessary.)
8) The glass slide was washed 3 times with PBS.
9) Add 100 µL of 1:500 FITC conjugated anti-mouse IgG diluted with PBS onto the cells. Incubate for 1 hour at room temperature. Keep out light by aluminum foil.
10) The glass slide was washed 3 times with PBS.
11) Incubate the cells with 1 µg/mL of propidium iodide (PI) for 15 minutes at room temperature.
12) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
13) Promptly add PermafluorTM aqueous mounting medium onto the slide, then put a cover slip on it.
(Positive control for Immunocytochemistry; U251)

Product images:



Immunocytochemical detection of phosphorylated Vimentin (Ser55) on formaldehyde fixed U251 cells with AM26448AF-N.



Western blot analysis of phosphorylated Vimentin (Ser55) in U251 cells, M phase (1) and interphase (2) using AM26448AF-N.