OriGene Technologies, Inc.
9620 Medical Center Drive, Ste 200
Rockville, MD 20850, US
Phone: +1-888-267-4436
https://www.origene.com
techsupport@origene.com
EU: info-de@origene.com
CN: techsupport@origene.cn

Product datasheet for AM26458AF-N

Vimentin (VIM) pSer71 Rat Monoclonal Antibody [Clone ID: TM71]

Product data:

Product Type:	Primary Antibodies
Clone Name:	TM71
Applications:	IF, WB
Recommended Dilution:	**Western blot:** 1 µg/ml for chemiluminescence detection system. **Immunocytochemistry:** 1 µg/ml. For details see protocol below.
Reactivity:	Human, Mouse, Rat
Host:	Rat
Isotype:	IgG2a
Clonality:	Monoclonal
Immunogen:	KLH conjugated phospho-peptide PV71
Specificity:	This antibody recognizes the site-specific phosphorylation of vimentin at Ser71.
Formulation:	PBS containing 50% glycerol, pH 7.2. No preservative is contained. State: Azide Free State: Liquid Ig fraction
Concentration:	lot specific
Purification:	Protein G 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

View online »

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.

Synonyms: VIM

Note: This product was originally produced by MBL International.

Protocol:

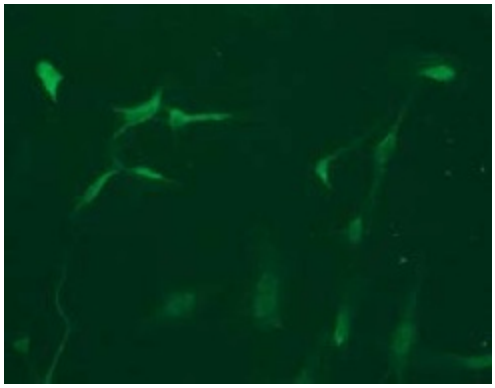
SDS-PAGE & Western Blotting

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 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 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] (5 minutes x 3 times).
9) Incubate the membrane with the 1:5,000 HRP-conjugated anti-rat 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.
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)

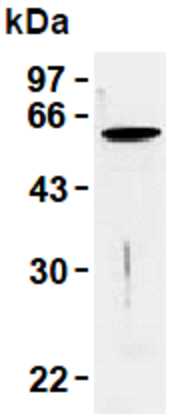
Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (for example, spread 1x10e4 of 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 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 are recommended if necessary.)
8) The glass slide was washed 3 times with PBS.
9) Add 100 µL of 1:100 FITC conjugated anti-rat IgG 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)

Product images:



Immunocytochemical detection of phosphorylated vimentin (Ser71) on 4% PFA fixed U251 cells with AM26458AF-N.



Western blot analysis of phosphorylated vimentin (Ser71) expression in U251 using AM26458AF-N.