

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Vimentin antibodies are ideal for immunocytochemistry colocalization studies in intermediate filaments. Detects human, mouse and rat Vimentin in Western blots.
Source	Monoclonal Rat IgG _{2A} Clone # 280618
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	<i>E. coli</i> -derived recombinant human Vimentin Ser2-Glu466 Accession # P08670
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

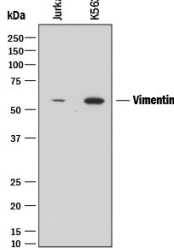
APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. *General Protocols* are available in the *Technical Information* section on our website.

	Recommended Concentration	Sample
Western Blot	1-2 µg/mL	See Below
Immunocytochemistry	8-25 µg/mL	See Below
Immunohistochemistry	0.5-25 µg/mL	See Below
Intracellular Staining by Flow Cytometry	0.25 µg/10 ⁶ cells	See Below
Simple Western	10 µg/mL	See Below
Knockout Validated	Vimentin is specifically detected in K562 human chronic myelogenous leukemia parental cell line but is not detectable in Vimentin knockout K562 cell line.	

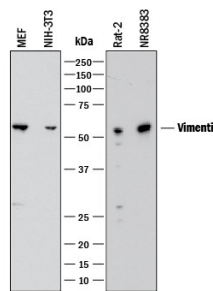
DATA

Western Blot



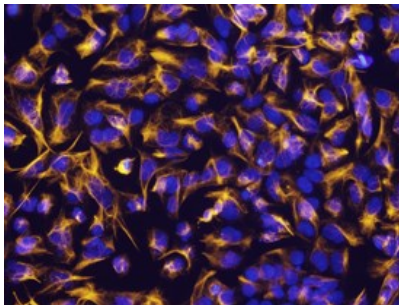
Detection of Human Vimentin by Western Blot. Western blot shows lysates of Jurkat human acute T cell leukemia cell line and K562 human chronic myelogenous leukemia cell line. PVDF membrane was probed with 2 µg/mL of Rat Anti-Human/Mouse/Rat Vimentin Monoclonal Antibody (Catalog # MAB2105) followed by HRP-conjugated Anti-Rat IgG Secondary Antibody (Catalog # HAF005). A specific band was detected for Vimentin at approximately 55 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Western Blot



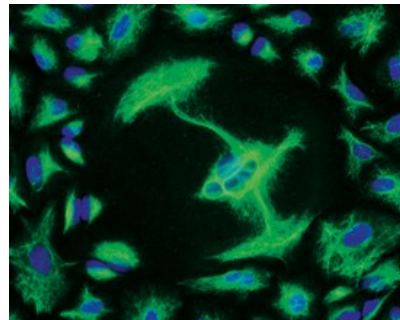
Detection of Mouse and Rat Vimentin by Western Blot. Western blot shows lysates of MEF mouse embryonic feeder cells, NIH-3T3 mouse embryonic fibroblast cell line, Rat-2 rat embryonic fibroblast cell line, and NR8383 rat alveolar macrophage cell line. PVDF membrane was probed with 1 µg/mL of Rat Anti-Human/Mouse/Rat Vimentin Monoclonal Antibody (Catalog # MAB2105) followed by HRP-conjugated Anti-Rat IgG Secondary Antibody (Catalog # HAF005). A specific band was detected for Vimentin at approximately 55 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry



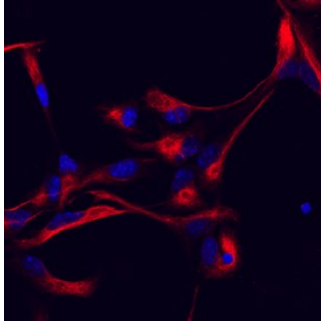
Vimentin in Ntera-2 Human Cell Line. Vimentin was detected in immersion fixed Ntera-2 human testicular embryonic carcinoma cell line using Rat Anti-Human/Mouse/Rat Vimentin Monoclonal Antibody (Catalog # MAB2105) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Rat IgG Secondary Antibody (yellow; Catalog # NL013) and counterstained with DAPI (blue). View our protocol for [Fluorescent ICC Staining of Cells on Coverslips](#).

Immunocytochemistry



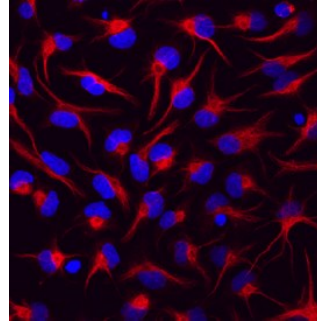
Vimentin in A549 Human Cell Line. Vimentin was detected in immersion fixed A549 human lung carcinoma cell line using Rat Anti-Human/Mouse/Rat Vimentin Monoclonal Antibody (Catalog # MAB2105) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 493-conjugated Anti-Rat IgG Secondary Antibody (green; Catalog # NL015) and counterstained with DAPI (blue). View our protocol for [Fluorescent ICC Staining of Cells on Coverslips](#).

Immunocytochemistry



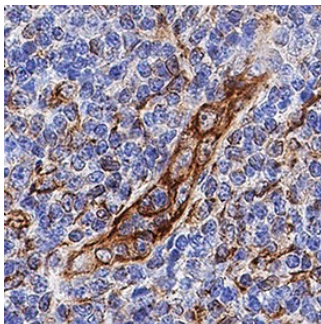
Vimentin in Mouse Cortical Stem Cells. Vimentin was detected in immersion fixed mouse cortical stem cells using Rat Anti-Human/Mouse/Rat Vimentin Monoclonal Antibody (Catalog # MAB2105) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Rat IgG Secondary Antibody (red; Catalog # Catalog # NL013) and counterstained with DAPI (blue). Specific staining was localized to cytoskeleton. View our protocol for [Fluorescent ICC Staining of Cells on Coverslips](#).

Immunocytochemistry



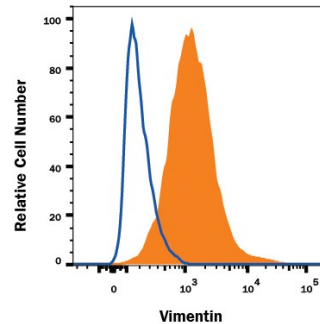
Vimentin in Rat Cortical Stem Cells. Vimentin was detected in immersion fixed rat cortical stem cells using Rat Anti-Human/Mouse/Rat Vimentin Monoclonal Antibody (Catalog # MAB2105) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Rat IgG Secondary Antibody (red; Catalog # Catalog # NL013) and counterstained with DAPI (blue). Specific staining was localized to cytoskeleton. View our protocol for [Fluorescent ICC Staining of Cells on Coverslips](#).

Immunohistochemistry



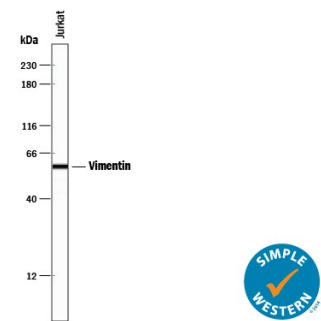
Vimentin in Human Tonsil. Vimentin was detected in immersion fixed paraffin-embedded sections of human tonsil using Rat Anti-Human/Mouse/Rat Vimentin Monoclonal Antibody (Catalog # MAB2105) at 0.5 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Rat IgG VisUCyte™ HRP Polymer Antibody (Catalog # Catalog # VC005). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to cytoplasm. View our protocol for [IHC Staining with VisUCyte HRP Polymer Detection Reagents](#).

Intracellular Staining by Flow Cytometry



Detection of Vimentin in A172 Human Cell Line by Flow Cytometry. A172 human glioblastoma cell line was stained with Rat Anti-Human/Mouse/Rat Vimentin Monoclonal Antibody (Catalog # MAB2105, filled histogram) or isotype control antibody (Catalog # Catalog # MAB006, open histogram) followed by anti-Rat IgG PE-conjugated secondary antibody (Catalog # Catalog # F0105B). To facilitate intracellular staining, cells were fixed with Flow Cytometry Fixation Buffer (Catalog # Catalog # FC004) and permeabilized with Flow Cytometry Permeabilization/Wash Buffer I (Catalog # Catalog # FC005). View our protocol for [Staining Intracellular Molecules](#).

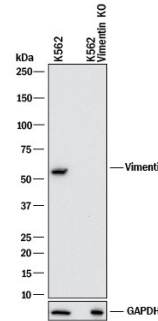
Simple Western



Detection of Human Vimentin by Simple Western™. Simple Western lane view shows lysates of Jurkat human acute T cell leukemia cell line, loaded at 0.2 mg/mL. A specific band was detected for Vimentin at approximately 58 kDa (as indicated) using 10 µg/mL of Rat Anti-Human/Mouse/Rat Vimentin Monoclonal Antibody (Catalog # MAB2105) followed by 1:50 dilution of HRP-conjugated Anti-Rat IgG Secondary Antibody (Catalog # Catalog # HAF005). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.



Knockout Validated



Western Blot Shows Human Vimentin Specificity by Using Knockout Cell Line. Western blot shows lysates of K562 human chronic myelogenous leukemia parental cell line and Vimentin knockout K562 cell line (KO). PVDF membrane was probed with 2 µg/mL of Rat Anti-Human/Mouse/Rat Vimentin Monoclonal Antibody (Catalog # MAB2105) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for Vimentin at approximately 55 kDa (as indicated) in the parental K562 cell line, but is not detectable in knockout K562 cell line. GAPDH (Catalog # MAB5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Western Blot Buffer Group 1.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <ul style="list-style-type: none"> • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

Vimentin is a 57 kDa class III intermediate filament (IF) protein that belongs to the intermediate filament family. It is the predominant IF in cells of mesenchymal origin such as vascular endothelium and blood cells (1-3). The human Vimentin cDNA encodes a 466 amino acid (aa) protein that contains head and tail regions with multiple regulatory Ser/Thr phosphorylation sites, and a central rod domain with three coiled-coil regions separated by linkers (1, 2). Human Vimentin shares 97-98% aa identity with mouse, rat, ovine, bovine, and canine Vimentin. Sixteen Vimentin coiled-coil dimers self-assemble to form intermediate (10-12 nm wide) filaments (4). These filaments then anneal longitudinally to form non-polarized fibers that support cell structure and withstand stress (4). IF fibers are highly dynamic, and half-life depends on the balance between kinase and phosphatase activity. For example, phosphorylation followed by dephosphorylation drives IF disintegration, followed by reorganization during mitosis (1, 5, 6). Interactions of head and tail domains link IFs with other structures such as actin and microtubule cytoskeletons (7). Vimentin is involved in positioning autophagosomes, lysosomes and the Golgi complex within the cell (8). It facilitates cell migration and motility by recycling internalized trailing edge integrins back to the cell surface at the leading edge (9-11). Vimentin helps maintain the lipid composition of cellular membranes, and caspase cleavage of Vimentin is a key event in apoptosis (8, 12). Phosphorylation promotes secretion of Vimentin by TNF- α -stimulated macrophages (13). Extracellular Vimentin has been shown to associate with several microbes, and appears to promote an antimicrobial oxidative burst (13, 14). Cell-associated Vimentin can also interact with NKp46 to recruit NK cells to tuberculosis-infected monocytes (15).

References:

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