Human Vimentin Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF2105

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human Vimentin in Western blots.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-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 as a 0.2 μm filtered solution in PBS.

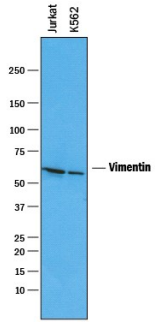
APPLICATIONS

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 µg/mL	See Below
Immunocytochemistry	5-15 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	See Below
Simple Western	10 µg/mL	See Below

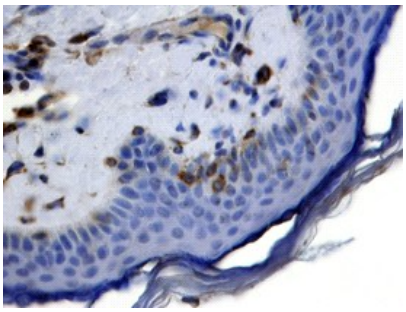
DATA

Western Blot



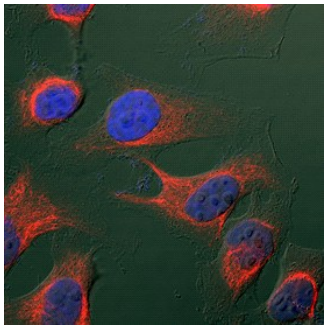
Detection of Human Vimentin by Western Blot. Western blot shows lysates of Jurkat human acute T cell leukemia cell line and K562 human chronic myelogenous leukemia cell line. PVDF membrane was probed with 1 µg/mL of Goat Anti-Human Vimentin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2105) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF019). 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.

Immunohistochemistry



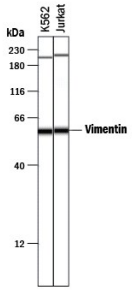
Vimentin in Human Skin. Vimentin was detected in immersion fixed paraffin-embedded sections of human skin using 10 µg/mL Goat Anti-Human Vimentin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2105) overnight at 4 °C. Tissue was stained with the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS008) and counterstained with hematoxylin (blue). View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

Immunocytochemistry



Vimentin in HeLa Human Cell Line. Vimentin was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line using Goat Anti-Human Vimentin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2105) at 1.7 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # NL001) and counterstained with DAPI (blue). Specific staining was localized to intermediate filaments in cytoplasm. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Simple Western



Detection of Human Vimentin by Simple Western™. Simple Western lane view shows lysates of K562 human chronic myelogenous leukemia cell line and Jurkat human acute T cell leukemia cell line, loaded at 0.2 mg/mL. A specific band was detected for Vimentin at approximately 59 kDa (as indicated) using 10 µg/mL of Goat Anti-Human Vimentin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2105) followed by 1:50 dilution of HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF109). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system. Non-specific interaction with the 230 kDa Simple Western standard may be seen with this antibody.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 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.** ● 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.

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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).

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