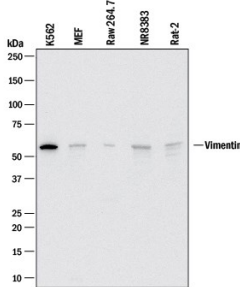
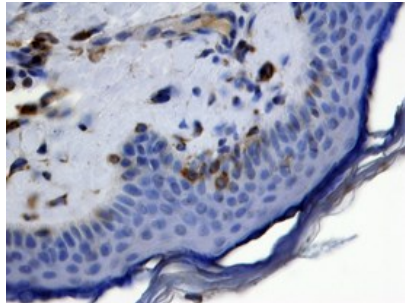
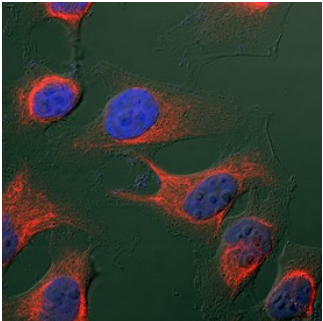


DESCRIPTION	
<b>Species Reactivity</b>	Human/Mouse/Rat
<b>Specificity</b>	Detects human, mouse, and rat Vimentin in Western blots.
<b>Source</b>	Polyclonal Goat IgG
<b>Purification</b>	Antigen Affinity-purified
<b>Immunogen</b>	<i>E. coli</i> -derived recombinant human Vimentin Ser2-Glu466 Accession # P08670
<b>Formulation</b>	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		
<b>Please Note:</b> Optimal dilutions should be determined by each laboratory for each application. <i>General Protocols</i> are available in the <i>Technical Information</i> section on our website.		
	Recommended Concentration	Sample
<b>Western Blot</b>	2 µg/mL	See Below
<b>Immunocytochemistry</b>	5-15 µg/mL	See Below
<b>Immunohistochemistry</b>	5-15 µg/mL	See Below
<b>Simple Western</b>	10 µg/mL	See Below
<b>Knockout Validated</b>	Vimentin is specifically detected in K562 human chronic myelogenous leukemia cell line but is not detectable in Vimentin knockout K562 cell line.	

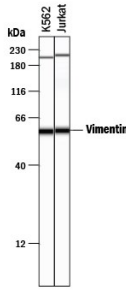
DATA	
<p><b>Western Blot</b></p>  <p><b>Detection of Human, Mouse, and Rat Vimentin by Western Blot.</b> Western blot shows lysates of K562 human chronic myelogenous leukemia cell line, MEF mouse embryonic feeder cells, RAW 264.7 mouse monocyte/macrophage cell line, NR8383 rat alveolar macrophage cell line, and Rat-2 rat embryonic fibroblast cell line. PVDF membrane was probed with 2 µg/mL of Goat Anti-Human/Mouse/Rat Vimentin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2105) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for Vimentin at approximately 55 kDa (as indicated). This experiment was conducted under reducing conditions and using Western Blot Buffer Group 1.</p>	<p><b>Immunohistochemistry</b></p>  <p><b>Vimentin in Human Skin.</b> Vimentin was detected in immersion fixed paraffin-embedded sections of human skin using 10 µg/mL Goat Anti-Human/Mouse/Rat Vimentin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2105) overnight at 4 °C. Tissue was stained with the Anti-Goat HRP-DAB Cell &amp; Tissue Staining Kit (brown; Catalog # CTS008) and counterstained with hematoxylin (blue). View our protocol for <a href="#">Chromogenic IHC Staining of Paraffin-embedded Tissue Sections</a>.</p>

**Immunocytochemistry**




**Vimentin in HeLa Human Cell Line.** Vimentin was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line using Goat Anti-Human/Mouse/Rat 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 # 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](#).

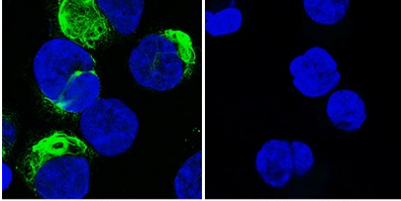
**Simple Western**



**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/Mouse/Rat Vimentin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2105) followed by 1:50 dilution of HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # 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.

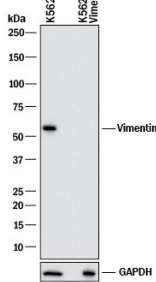


**Knockout Validated**



**Vimentin Specificity is Shown by Immunocytochemistry in Knockout Cell Line.** Vimentin was detected in immersion fixed K562 human chronic myelogenous leukemia cell line but is not detected in Vimentin knockout (KO) K562 Human Cell Line cell line using Goat Anti-Human/Mouse/Rat Vimentin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2105) at 5 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 493-conjugated Anti-Goat IgG Secondary Antibody (green; Catalog # Catalog # NL003) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. Staining was performed using our [Fluorescent ICC Staining of Non-adherent Cells](#) protocol.

**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 Goat Anti-Human/Mouse/Rat Vimentin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2105) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # 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 # AF5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Western Blot Buffer Group 1.

**PREPARATION AND STORAGE**

<b>Reconstitution</b>	Reconstitute at 0.2 mg/mL in sterile PBS.
<b>Shipping</b>	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
<b>Stability &amp; Storage</b>	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <ul style="list-style-type: none"> <li>• 12 months from date of receipt, -20 to -70 °C as supplied.</li> <li>• 1 month, 2 to 8 °C under sterile conditions after reconstitution.</li> <li>• 6 months, -20 to -70 °C under sterile conditions after reconstitution.</li> </ul>

**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- $\alpha$ -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:**

1. Omary, M.B. *et al.* (2006) Trends Biochem. Sci. **31**:383.
2. Ivaska, J. *et al.* (2007) Exp. Cell Res. **313**:2050.
3. Ferrari, S. *et al.* (1986) Mol. Cell. Biol. **6**:3614.
4. Sokolova, A.V. *et al.* (2006) Proc. Natl. Acad. Sci. USA **103**:16206.
5. Eriksson, J.E. *et al.* (2004) J. Cell Sci. **117**:919.
6. Li, Q.-F. *et al.* (2006) J. Biol. Chem. **281**:34716.
7. Esue, O. *et al.* (2006) J. Biol. Chem. **281**:30393.
8. Styers, M.L. *et al.* (2005) Traffic **6**:359.
9. McInroy, L. and A. Maata (2007) Biochem. Biophys. Res. Commun. **360**:109.
10. Nieminen, M. *et al.* (2006) Nat. Cell Biol. **8**:156.
11. Ivaska, J. *et al.* (2005) EMBO J. **24**:3834.
12. Byun, Y. *et al.* (2001) Cell Death Differ. **8**:443.
13. Mor-Vaknin, N. *et al.* (2003) Nat. Cell Biol. **5**:59.
14. Zou, Y. *et al.* (2006) Biochem. Biophys. Res. Commun. **351**:625.
15. Garg, A. *et al.* (2006) J. Immunol. **177**:6192.