

DESCRIPTION

Species Reactivity	Human/Rat
Specificity	Detects recombinant human Vinculin in direct ELISAs and human and rat Vinculin in Western blots.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	<i>E. coli</i> -derived recombinant human Vinculin Lys1020-Gln1134 Accession # P18206
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
Immunohistochemistry	5-15 µg/mL	See Below

DATA

<p>Western Blot</p> <p>Detection of Human and Rat Vinculin by Western Blot. Western blot shows lysates of HeLa human cervical epithelial carcinoma cell line and rat heart tissue. PVDF membrane was probed with 1 µg/mL of Sheep Anti-Human/Rat Vinculin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6896) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for Vinculin at approximately 120 kDa (as indicated). This experiment was conducted under reducing conditions and using <i>Immunoblot Buffer Group 1</i>.</p>	<p>Immunohistochemistry</p> <p>Vinculin in Human Uterus. Vinculin was detected in immersion fixed paraffin-embedded sections of human uterus using Sheep Anti-Human/Rat Vinculin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6896) at 3 µg/mL overnight at 4 °C. Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). Tissue was stained using the Anti-Sheep HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS019) and counter-stained with hematoxylin (blue). View our protocol for <i>Chromogenic IHC Staining of Paraffin-embedded Tissue Sections</i>.</p>
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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. <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

VCL (Vinculin; from the Latin *vinculum* denoting a union or connection) is a 115-120 kDa cytoplasmic member of the vinculin/α-catenin family of proteins. The VCL gene is ubiquitously expressed and generates a series of globular domains whose N- and C-terminal globular regions self-associate to generate an inactive molecule. When at least two ligands bind to the molecule, it opens up, allowing for the formation of multiple complexes that impact both signaling and cell structure. Among other things, VCL is known to regulate the expression of E-cadherin, and to connect actin to integrins via talin. Human VCL is 1134 amino acids (aa) in length, and at this size, is commonly known as metavinculin. It contains an 835 aa N-terminal globular head that binds talin, β- and α-catenin, followed by a Pro-rich linker segment (aa 836-878) and a 27-29 kDa C-terminal tail that binds lipids plus paxillin and PKCα (aa 879-1134). At least seven utilized phosphorylation sites exist. There are three potential splice variants. One shows a deletion of aa 916-983 and is called vinculin. A second contains an alternative start site at Met74, and again possesses a deletion of aa 916-983. A third shows a 34 aa substitution for aa 262-1134. Over the range of aa used as the immunogen for this reagent (aa 1020-1134 in metavinculin; 952-1066 in vinculin), human and mouse VCL are identical in aa sequence.