

### VHL Antibody (N-term)

Purified Rabbit Polyclonal Antibody (Pab) Catalog # AP6549A

### Specification

# VHL Antibody (N-term) - Product Information

Application Primary Accession Other Accession Reactivity Predicted Host Clonality Isotype Antigen Region WB, IF, IHC-P, FC,E <u>P40337</u> <u>O64259</u>, <u>P40338</u> Human, Mouse Rat Rabbit Polyclonal Rabbit IgG 43-71

# VHL Antibody (N-term) - Additional Information

Gene ID 7428

Other Names Von Hippel-Lindau disease tumor suppressor, Protein G7, pVHL, VHL

#### Target/Specificity

This VHL antibody is generated from rabbits immunized with a KLH conjugated synthetic peptide between 43-71 amino acids from the N-terminal region of human VHL.

**Dilution** WB~~1:2000 IF~~1:25 IHC-P~~1:25 FC~~1:25

Format

Purified polyclonal antibody supplied in PBS with 0.09% (W/V) sodium azide. This antibody is purified through a protein A column, followed by peptide affinity purification.

#### Storage

Maintain refrigerated at 2-8°C for up to 2 weeks. For long term storage store at -20°C in small aliquots to prevent freeze-thaw cycles.

#### **Precautions**

VHL Antibody (N-term) is for research use only and not for use in diagnostic or therapeutic procedures.

#### VHL Antibody (N-term) - Protein Information

Name VHL



**Function** Involved in the ubiquitination and subsequent proteasomal degradation via the von Hippel-Lindau ubiquitination complex (PubMed:<u>10944113</u>, PubMed:<u>17981124</u>, PubMed:<u>19584355</u>). Seems to act as a target recruitment subunit in the E3 ubiquitin ligase complex and recruits hydroxylated hypoxia-inducible factor (HIF) under normoxic conditions (PubMed:<u>10944113</u>, PubMed:<u>17981124</u>). Involved in transcriptional repression through interaction with HIF1A, HIF1AN and histone deacetylases (PubMed:<u>10944113</u>, PubMed:<u>17981124</u>). Ubiquitinates, in an oxygen-responsive manner, ADRB2 (PubMed:<u>19584355</u>). Acts as a negative regulator of mTORC1 by promoting ubiquitination and degradation of RPTOR (PubMed:<u>34290272</u>).

#### **Cellular Location**

[Isoform 1]: Cytoplasm. Cell membrane; Peripheral membrane protein. Endoplasmic reticulum. Nucleus. Note=Found predominantly in the cytoplasm and with less amounts nuclear or membrane-associated (PubMed:9751722) Colocalizes with ADRB2 at the cell membrane (PubMed:19584355)

**Tissue Location** 

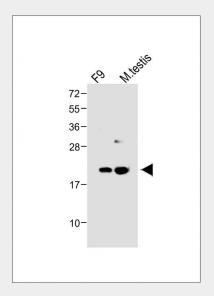
Expressed in the adult and fetal brain and kidney.

### VHL Antibody (N-term) - Protocols

Provided below are standard protocols that you may find useful for product applications.

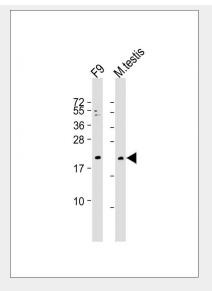
- <u>Western Blot</u>
- Blocking Peptides
- Dot Blot
- Immunohistochemistry
- Immunofluorescence
- Immunoprecipitation
- Flow Cytomety
- <u>Cell Culture</u>

#### VHL Antibody (N-term) - Images

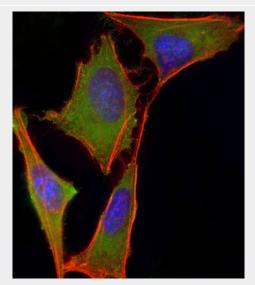


All lanes : Anti-VHL Antibody (N-term) at 1:1000 dilution Lane 1: F9 whole cell lysate Lane 2: Mouse testis lysate Lysates/proteins at 20  $\mu$ g per lane. Secondary Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/10000 dilution. Predicted band size : 24 kDa Blocking/Dilution buffer: 5% NFDM/TBST.

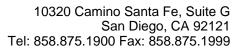




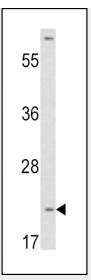
All lanes : Anti-VHL Antibody (N-term) at 1:500 dilution Lane 1: F9 whole cell lysate Lane 2: Mouse testis lysate Lysates/proteins at 20  $\mu$ g per lane. Secondary Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/10000 dilution. Predicted band size : 24 kDa Blocking/Dilution buffer: 5% NFDM/TBST.



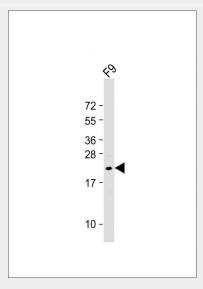
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (human cervical epithelial adenocarcinoma cell line) cells labeling VHL with AP6549a at 1/25 dilution, followed by Dylight® 488-conjugated goat anti-rabbit IgG (NK179883) secondary antibody at 1/200 dilution (green). Immunofluorescence image showing nucleus and cytoplasm staining on HeLa cell line. Cytoplasmic actin is detected with Dylight® 554 Phalloidin (PD18466410) at 1/100 dilution (red). The nuclear counter stain is DAPI (blue).





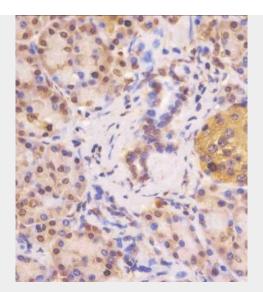


Western blot analysis of VHL antibody (N-term) (Cat. #AP6549a) in HepG2 cell line lysates (35ug/lane). VHL (arrow) was detected using the purified Pab.

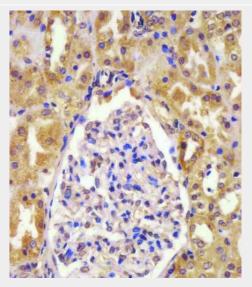


Anti-VHL Antibody (N-term) at 1:2000 dilution + F9 whole cell lysate Lysates/proteins at 20 µg per lane. Secondary Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/10000 dilution. Predicted band size : 24 kDa Blocking/Dilution buffer: 5% NFDM/TBST.



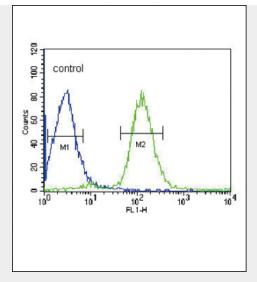


AP6549a staining VHL in human pancreas tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with formaldehyde and blocked with 3% BSA for 0. 5 hour at room temperature; antigen retrieval was by heat mediation with a citrate buffer (pH6). Samples were incubated with primary antibody (1/25) for 1 hours at 37°C. A undiluted biotinylated goat polyvalent antibody was used as the secondary antibody.

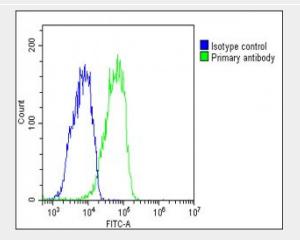


AP6549a staining VHL in human kidney tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with formaldehyde and blocked with 3% BSA for 0. 5 hour at room temperature; antigen retrieval was by heat mediation with a citrate buffer (pH6). Samples were incubated with primary antibody (1/25) for 1 hours at 37°C. A undiluted biotinylated goat polyvalent antibody was used as the secondary antibody.





VHL Antibody (N-term) (Cat. #AP6549a) flow cytometric analysis of HepG2 cells (right histogram) compared to a negative control cell (left histogram).FITC-conjugated goat-anti-rabbit secondary antibodies were used for the analysis.



Overlay histogram showing HepG2 cells stained with AP6549a(green line). The cells were fixed with 2% paraformaldehyde (10 min) and then permeabilized with 90% methanol for 10 min. The cells were then icubated in 2% bovine serum albumin to block non-specific protein-protein interactions followed by the antibody (AP6549a, 1:25 dilution) for 60 min at 37°C. The secondary antibody Goat-Anti-Rabbit **DyLight**® 488 used was lgG, Conjugated Highly Cross-Adsorbed(1583138) at 1/200 dilution for 40 min at 37°C. Isotype control antibody (blue line) was rabbit  $IgG1 (1\mu g/1x10^6 \text{ cells})$  used under the same conditions. Acquisition of >10, 000 events was performed.

# VHL Antibody (N-term) - Background

Von Hippel-Lindau syndrome (VHL) is a dominantly inherited familial cancer syndrome predisposing to a variety of malignant and benign tumors. A germline mutation of VHL gene is the basis of familial inheritance of VHL syndrome. The protein is a component of the protein complex that includes elongin B, elongin C, and cullin-2, and possesses ubiquitin ligase E3 activity. This protein is involved in the ubiquitination and degradation of hypoxia-inducible-factor (HIF), which is a transcription factor that plays a central role in the regulation of gene expression by oxygen. RNA polymerase II subunit POLR2G/RPB7 is also reported to be a target of this protein.

# VHL Antibody (N-term) - References



Olmos,G., Cell. Mol. Life Sci. 66 (13), 2167-2180 (2009) Hatzimichael,E., Clin Lymphoma Myeloma 9 (3), 239-242 (2009) Luu,V.D., Clin. Cancer Res. 15 (10), 3297-3304 (2009)