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## Antibodies to Viral Proteins

### Viral Protein U (Vpu) of Human Immunodeficiency virus (Cat # Vpu-101AP and P-Vpu)

*Alternate Nomenclature: HIV 1 Vpu protein*

Retroviruses have several characteristic structural and catalytic proteins, one such auxiliary protein is a viral protein U (Vpu) which enhances virion release from human cells and also involved in the degradation of CD4, the cellular surface receptor of HIV-1. The Vpu has no homolog in less pathogenic HIV-2 virus. Vpu is an 81 amino acid class I membrane integral protein that is unique to human and simian immunodeficiency virus isolated from Chimpanzee and few other monkey species. The 16kDa protein Vpu protein consist of an N-terminal hydrophobic membrane anchor of 27 amino acids and a charged C-terminal hydrophilic domain of 54 amino acids that extends to the cytoplasm. The cytoplasmic domain has a conserved dual serine phosphorylation site (S52 GXX & S56 motif) that is phosphorylated by casein kinase II (1). Vpu is involved in viral replication an degradation of its cellular receptor CD4 and enhancement of viral particle release from macrophages and primary lymphocytes. The degradation of CD4 receptor is achieved by hijacking of protein degradation machinery of the host cells that involves ubiquitin ligases that ensures the selection of proteins to be degraded. Vpu binds to CD4 and simultaneously recruits the  $\beta$ TrCP subunit of the SCF <sup>$\beta$ TrCP</sup> ubiquitin ligase complex through its constitutively phosphorylated DS<sub>52</sub>GXXS<sub>56</sub> motif. In this process, Vpu was found to escape degradation, while inhibiting the degradation of  $\beta$ TrCP natural targets such as  $\beta$ -catenin and I $\kappa$ Ba (2). Interestingly, the Vpu activity was not observed in simian cells probably due to its ability to counter act host cell restriction factor specific for human cells and may depend on Vpu binding to host channel TASK-1 protein (3). Vpu is degraded in cells arrested in early mitosis by nocodazole, the degradation process require phosphorylation of the serine 61 residue adjacent to the  $\beta$ TrCP-binding motif (3). Vpu has all the characteristics of signal peptide sequences (hydrophobic N-terminal and a hydrophilic C-terminal tail) when cleaved by signal peptidases stays with lipids of the signal peptidase complex, after further processing the N-terminal region is released into cytosol where it interacts with calmodulin and preprolactin.

The Minpp1-selective antibodies were generated against synthetic peptide corresponding to residues 26-46 of the human Minpp1 (the peptide sequence is conserved in rat Minpp1). The antibodies to Minpp1 are affinity purified over immobilized antigen based affinity chromatography. The purified antibodies are stabilized in antibody stabilization buffer containing preservatives. FabGennix Int. Inc., also provide western blot positive control for Minpp1 in ready-to-use buffer and limited quantities of antigenic blocking peptide is also available, please inquire about pricing and availability. FabGennix also carries many antibodies to receptor and non-receptor kinases and phosphatase, for a complete listing please visit [www.FabGennix.com](http://www.FabGennix.com). FabGennix Inc. will also conjugate antibodies with fluorescent probes upon request at a reasonable cost.

Catalog #	Host Species	Nature	Cross reactivity	Quantity	Vol
Vpu-101AP	Rabbit	Affinity purified Vpu antibodies	HIV 1	100 ug	200ul
FITC-Vpu	Rabbit	FITC conjugated affinity purified Vpu antibody	HIV-1	100ug	200ul
P-Vpu	n/a	Antigenic blocking peptide for Vpu-101AP	n/a	250ug	100ul

R = rat; M = mouse; H = human; C = chicken; monk = monkey; \* not all variants are labeled equally

**Immunogen:** Synthetic peptide from amino acids 58-80 corresponding to Vpu peptide (amino acid seq: egd qee lsa lme mgh hap wnv nd), was selected from the unique region of the Vpu protein, peptide was post-synthetically modified to achieve highest antigenicity before used for coupling to KLH using heterobifunctional cross linker for immunogen preparation.

**Concentration:** Vpu-101AP Ig concentration 0.63-0.68 mg/ml in antibody stabilization buffer. Concentration of FITC-Vpu is 0.51-0.55mg/ml in antibody stabilization buffer.

**Applications:** Antibody Vpu-101AP is characterized by ELISA using antigenic blocking peptide. The antibody titer for antigenic peptide is 1:100k. We have tested this antibody on westerns or for IF applications using HEK293 cells transfected with HIV-1 Vpu cDNA at 1:200 and 1:500 dilutions. The dilutions for this antibody is for reference only, investigators are expected to determine the optimal conditions in their laboratory. ELISA: 1:50K; Western blotting 1:1000; Immunoprecipitation 1:250; IHC/IF = 1:250. Investigators who want to use this antibody in applications not listed here can ask for a complimentary sample of Vpu-101AP antibodies. We will be happy to provide this antibody form multiple rabbits.

**Protocols:** Standard protocol for various applications (WB; IMM and IHC) of this antibody is provided with the product specification sheet, however, FabGennix Inc. strongly recommends investigators to optimize conditions for use of this antibody in their laboratories.

**Form/Storage:** The antiserum is supplied in antibody stabilization buffer with 0.02% sodium azide as preservatives. The affinity-purified antibodies are purified on antigen-sepharose affinity column and supplied as stabilized antibody. For long-term storage of antibodies, store at -20°C. FabGennix Inc. does not recommend storage of very dilute antibody solutions unless they are prepared in specially formulated multi use antibody dilution buffer (Cat # DiluOBuffer). Working solutions of antibodies in DiluOBuffer should be filtered through 0.45  $\mu$  filter after every use for long-term storage.

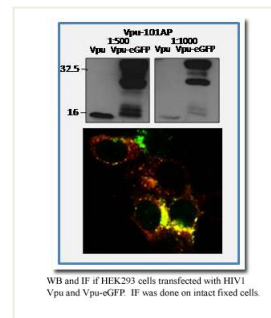
**Note:** Now you can recycle your western blots (nitrocellulose, supported membranes and PVDF membranes) by using our StripOBuffer (Cat FGI-1989). Each stripping is guaranteed to give better signal (up to 8 stripping). No strong pungent smell of reducing agents or heating is required.

### References:

1. Julie Binette., Mathieu Dube., Johanne Mercier., et al., Requirement for the selective degradation of CD4 receptor molecule by the human immunodeficiency virus type 1 Vpu protein in endoplasmic reticulum. *Retrovirology*, 2007. 4. 75.
2. Emilie Estrabaud, Erwann Le Rouzic, Sandra Lopez-Vergès, Marina Morel, Nadia Belaidouni, Richard Benarous, Catherine Transy, Clarisse Berlioz-Torrent, and Florence Margottin-Goguet. Regulated Degradation of the HIV-1 Vpu Protein through a  $\beta$ TrCP-Independent Pathway Limits the Release of Viral Particles. *PLoS Pathog.* 2007 July; 3(7): e104.
3. Hsu K, Seharaseyon J, Dong P, Bour S, Marban E. Mutual functional destruction of HIV-1 Vpu and host TASK-1 channel. *Mol Cell.* 2004;14:259-267.

\*For users who may require large amounts of Vpu-101AP and FITC-Vpu, please enquire about bulk material discounts.  
This Product is for Research Use Only and is NOT intended for use in humans or clinical diagnosis.

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