Anti-Ubiquitin Antibody, Mouse monoclonal, VU-1

Cat. # VU101

Background:

Ubiquitin (Ub), a highly conserved, 8kDa polypeptide present in all eukaryotic cells, is conjugated to the ε-amino group of lysine residues in the target protein through the sequential action of three enzymes, an E1 Ub activating enzyme, an E2 conjugating enzyme, and an E3 ligase. In addition, the seven lysines within Ub itself can serve as Ub acceptors leading to the formation of polyubiquitin chains. Among these, K48 and K63 linkages are well characterized. K48-linked polyubiquitylation targets proteins for proteasome degradation whereas K63 linkages regulate signaling events, receptor endocytosis and immune responses. Polyubiquitin linkages at other lysines are less prominent and their physiological role remains largely unknown.

LifeSensors has developed the VU-1 ubiquitin monoclonal antibody that recognize poly- and monoubiquitylated proteins and free ubiquitin. VU-1 has been shown to recognize K48-, K63-, K11-linkages as well as linear ubiquitin chains; other linkages have not been tested.

Applications:

Western blotting (1:1000) PLEASE SEE THE PROTOCOL BELOW ELISA, IHC (1:200 – 1:500)

For all applications, optimal conditions should be determined by the end user.

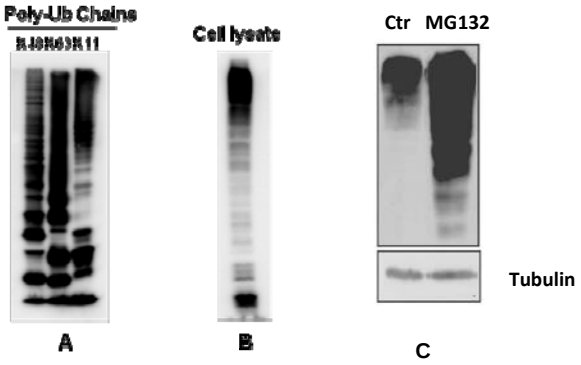
Product Information

Source:	Purified from hybridoma supernatant	**Isotype:**	IgG1 (murine)
Purity:	≥ 90%	**Clone:**	VU-1
Supplied as:	0.5mg/ml in PBS, pH 7.2		

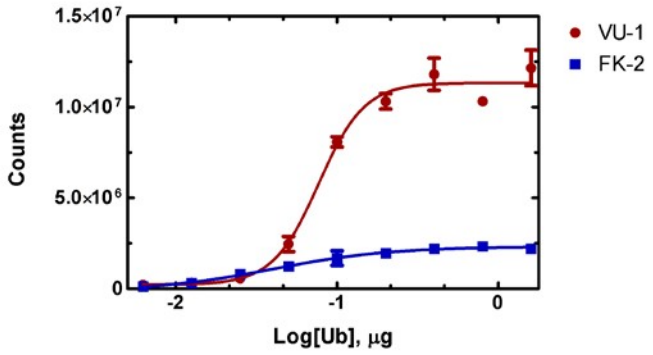
Specificity: Antibody detects proteins modified by poly- and monoubiquitin, linear ubiquitin chains, and free ubiquitin. Reactivity is independent of species due to the high conservation of ubiquitin across eukaryotes

Quantity: 250 µg. Sufficient for >50 Western blot applications

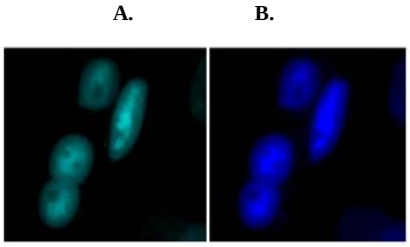
Storage: Store at -20°C. Avoid multiple freeze/thaw cycles.



Western blot analysis of chain-specific and cellular ubiquitylated conjugates using Mab VU-1. (A). K48, K63 and K11-linked polyubiquitin conjugates; **(B).** 40 µg HEK293T cell lysate (50 nM Bortezomib treated); **(C)**.OLN cells untreated (Ctr) and treated with 1 µM MG132. Cell lysates were separated on SDS-PAGE and immunoblotted with VU-1 antibody (**B**.1 µg/ml; **C**.0.2 µg/ml).

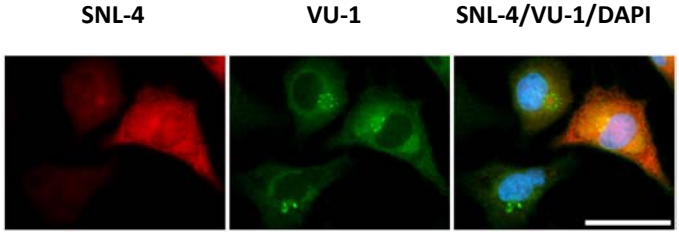


VU-1 monoclonal antibody detects unconjugated ubiquitin in ELISA. Unconjugated ubiquitin was coated on polystyrene plates, blocked and incubated with either VU-1 or FK2 antibodies (1µg/ml) followed by anti-mouse HRP conjugate (1:5000). After adding ECL the plate was read using Envision plate reader.

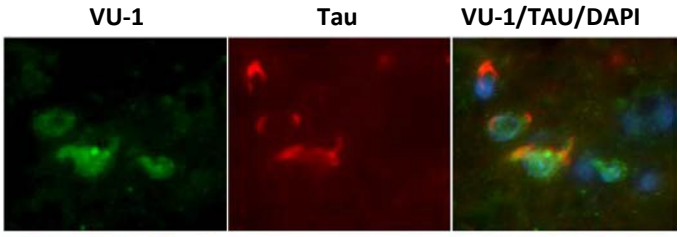


VU-1 immunostaining is co-localized with PABPN1 aggregates in human myoblasts. Human myoblasts were transfected with PABPN1-YFP that was shown previously to form ubiquitylated nuclear aggregates. **A**. Nuclear localization of PABPN1-YFP in myoblasts. **B.** VU-1 co-localization with PABPN1.

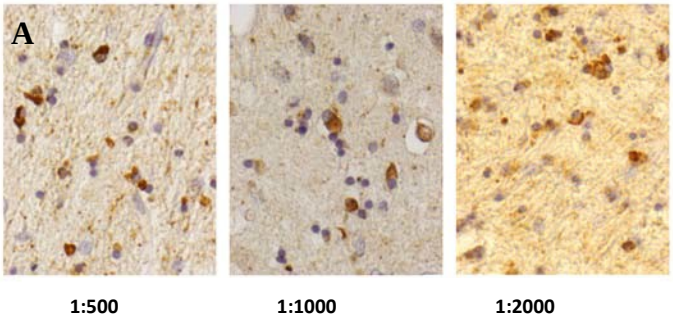
Images are kindly provided by V.Raz, Ph.D, Dept of Human Genetics, Leiden University, The Netherlands.



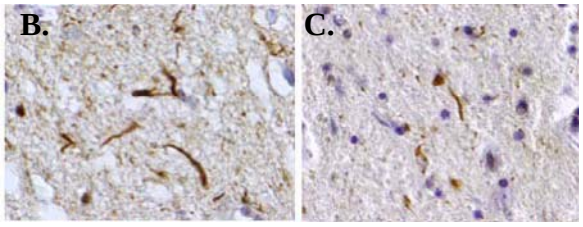
VU-1 immunoreactivity in OLN93 cell line expressing α-synuclein. Cells were treated with 1 μM of MG132 for 2 h and fixed in methanol. Cytoplasmic α-synuclein-positive, red (SNL-4) and Ubiquitin-positive inclusions, green (VU-1, dilution 1:200).



Ubiquitin-positive (VU-1) and Tau-labeled neurons in human brain tissue (Progressive Supranuclear Palsy, PSP). Paraffin embedded, formalin fixed 6 μm sections were stained with MAb VU-1 (1:1000) after antigen retrieval. To block autofluorescence, tissue sections were treated with Sudan black.



Ubiquitin-positive (VU-1) inclusions in human brain. A. MSA (Multiple System Atrophy,);**B**. PD (Parkinson's); **C**.PSP (Progressive Supranuclear Palsy). Paraffin embedded, formalin fixed 3 μm tissue section (pons) were stained with VU-1 MAb at different dilutions. DAB staining protocol was used to visualize the inclusions.



Images are kindly provided by Prof.C.Richter-Landsberg, Molekulare Neurobiologie, Carl von Ossietzky Universität, Oldenburg, Germany

Suggested Protocols

Western blot analysis

1. Prepare samples in the buffer of choice in the presence of protease inhibitors and 50 μM PR619 (cat.no.SI9619)*.
2. Run the gel and transfer onto PVDF membrane.Wash membrane with PBS or H_2O 3 x 2 min.
3. **Pretreatment: Incubate membrane with 0.5% glutaraldehyde/PBS pH 7.0 for 20 min.**
   Important Note: DO NOT USE Tris-HCl containing buffer at this step since glutaraldehyde is amine reactive.
4. Wash membrane 3 x PBS
5. Block in 5% non-fat milk/TBS/0.1%Tween (TBST) for 30 min. at room temperature.
6. Incubate with primary antibody VU-1 (1 μg/ml) at 4^{o} overnight.
7. Wash membrane with TBST 2 x 10 min.
8. Incubate with secondary HRP labeled mouse IgG of choice. Tested with Jackson ImmunoResearch, cat.no. 715-035-150)
9. Develop using ECL of choice. Tested with Thermo ECL, cat.no. 34080.

*PR619, a small molecule, cell permeable, non-selective DUB inhibitor, to prevent deubiquitylation of protein during cell lysis.

References

1 Wilkinson, K.D., Regulation of ubiquitin-dependent processes by deubiquitinating enzymes. *Faseb J*, 1997. **11:**1245-56.

2 Meulmeester E, et al Loss of HAUSP-mediated deubiquitination contributes to DNA damage-induced destabilization of Hdmx and Hdm2. *Mol Cell*. 2005 **18:**565-76.

3 Faustrup H, et al. USP7 counteracts SCFbetaTrCP- but not APCCdh1-mediated proteolysis of Claspin. *J Cell Biol.* 2009 **184:**13-9.

4 Li M,et al. Deubiquitination of p53 by HAUSP is an important pathway for p53 stabilization *Nature*, 2002 **416:**648-53.

5. Seiberlich V. et.al. The small molecule inhibitor PR-619 of deubiquitinating enzymes affects the microtubule network and causes protein aggregate formation in neural cells: Implications for neurodegenerative diseases. *Biochem et Biophys Acta*, 2012, 1823;2057-2068.