Anti-Ubiquitin, HRP-Conjugated (MAb, Clone VU-1)

Cat. # VU101H

Background:

LifeSensors has developed several monoclonal antibodies that recognize polyubiquitylated proteins and free ubiquitin. VU-1 recognizes all ubiquitin linkages (mono, K6, K11, K27, K29, K33, K48, K63, and linear) and now we have conjugated VU-1 to HRP so western blotting is performed in a single step (no secondary antibody required).

Ubiquitin (Ub), a highly conserved, 8 kDa 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 roles are under investigation.

Applications:

Western blotting (1:2000) **Note: Best results observed by treating membrane with glutaraldehyde**.

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

Product Information

Source	**Purified from hybridoma supernatant**
Purity:	**≥ 90%**
Specificity	**Antibody detects mono-Ub and all types of poly-Ub linkages**
Clone	**VU-1**
Isotype	**IgG1 (mouse)**
Cross Reactivity	**Human.** Reactivity against ubiquitin from other species is likely due to the high conservation between these orthologs and antigen used for the antibody production
Quantity:	50 μg. Enough for >20 Western blot applications; Glutaraldehyde solution included
Storage:	Store at -20°C. Avoid multiple freeze/thaw cycles.

Western blot analysis

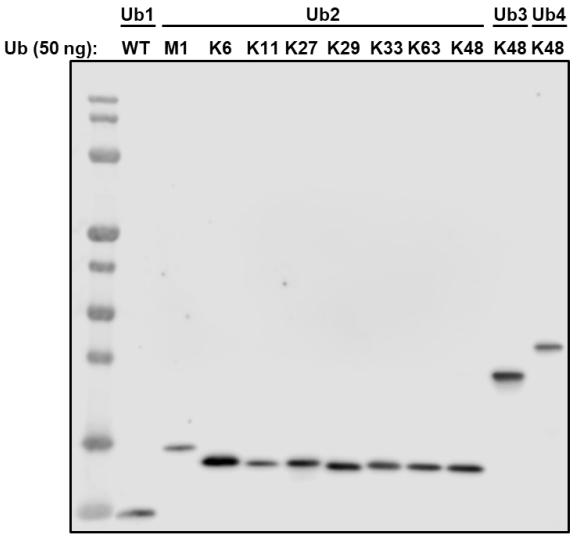
1. Run the gel and transfer onto PVDF or nitrocellulose membrane.
2. Wash membrane with PBS or H_2O 3 x 2 min.
3. **Incubate membrane with 0.5% glutaraldehyde/PBS pH 7.0 for 20 min**

Important Note: ***DO NOT USE*** *Tris-HCl containing buffer since glutaraldehyde is amine reactive.*

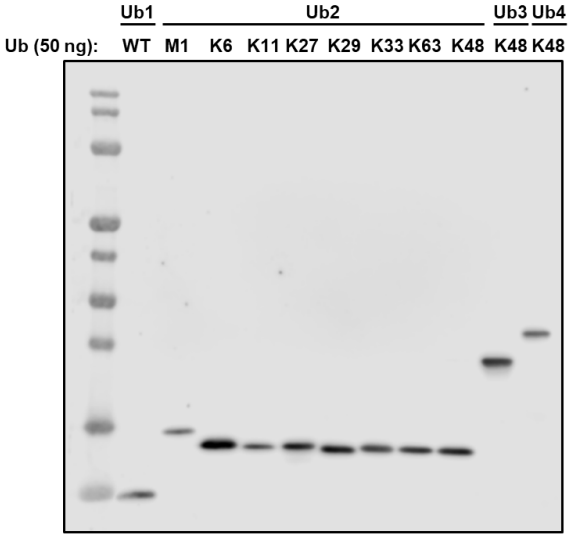
4. Wash membrane with PBS 3 x 10 min
5. Blocking buffer: 5% non-fat milk/TBS/0.1%Tween (TBST); for 30 min at room temperature.
6. Incubate with VU-1 HRP (1:2000 dilution in blocking buffer) for 2 h at room temperature.
7. Wash membrane with TBST 3 x 10 min.
8. Develop using ECL of choice. Tested with Thermo ECL, cat.no. 34080.

References

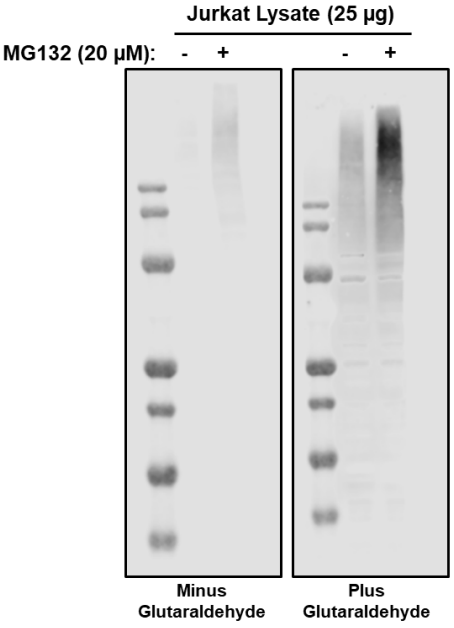
1. Oh E, Akopian D, Rape M. Principles of Ubiquitin-Dependent Signaling. *Annu. Rev. Cell Dev. Biol.* 2018;34(1):137–162.
2. Akutsu M, Dikic I, Bremm A. Ubiquitin chain diversity at a glance. *J. Cell Sci.* 2016;129(5):875–880.
3. Komander D, Rape M. The Ubiquitin Code. *Annu. Rev. Biochem.* 2012;81(1):203–229.



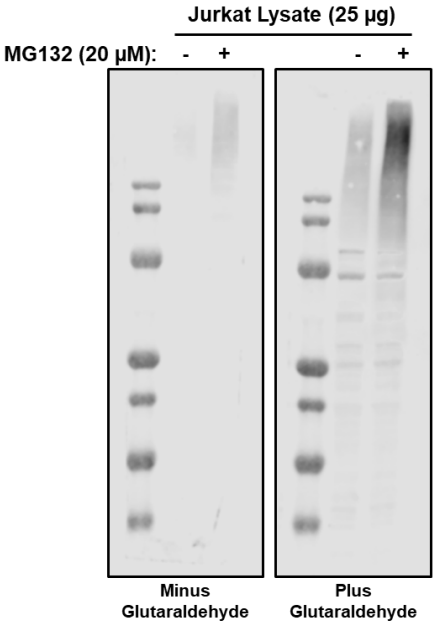
IB: VU-1 HRP (1:2000 dilution)
(2.5 µg/10 ml)



IB: VU-1 (1:500 dilution)
(10 µg/10 ml)



IB: VU-1 HRP (1:2000 dilution)
(2.5 µg/10 ml)



IB: VU-1 (1:500 dilution)
(10 µg/10 ml)