

## Anti-Ub TUBE2, HRP

Cat. # UM312

**Background:** Based on protein domains known to possess an affinity for ubiquitin, Tandem Ubiquitin Binding Entities (TUBEs) have been developed for the isolation and identification of ubiquitylated proteins. TUBEs display up to a 1000-fold increase in affinity for poly-ubiquitin moieties over the single ubiquitin binding associated domain (UBA). In addition, TUBEs display a protective effect on polyubiquitinated proteins, allowing for detection at relatively low abundance. These properties effectively "capture" proteins in their polyubiquitylated state.

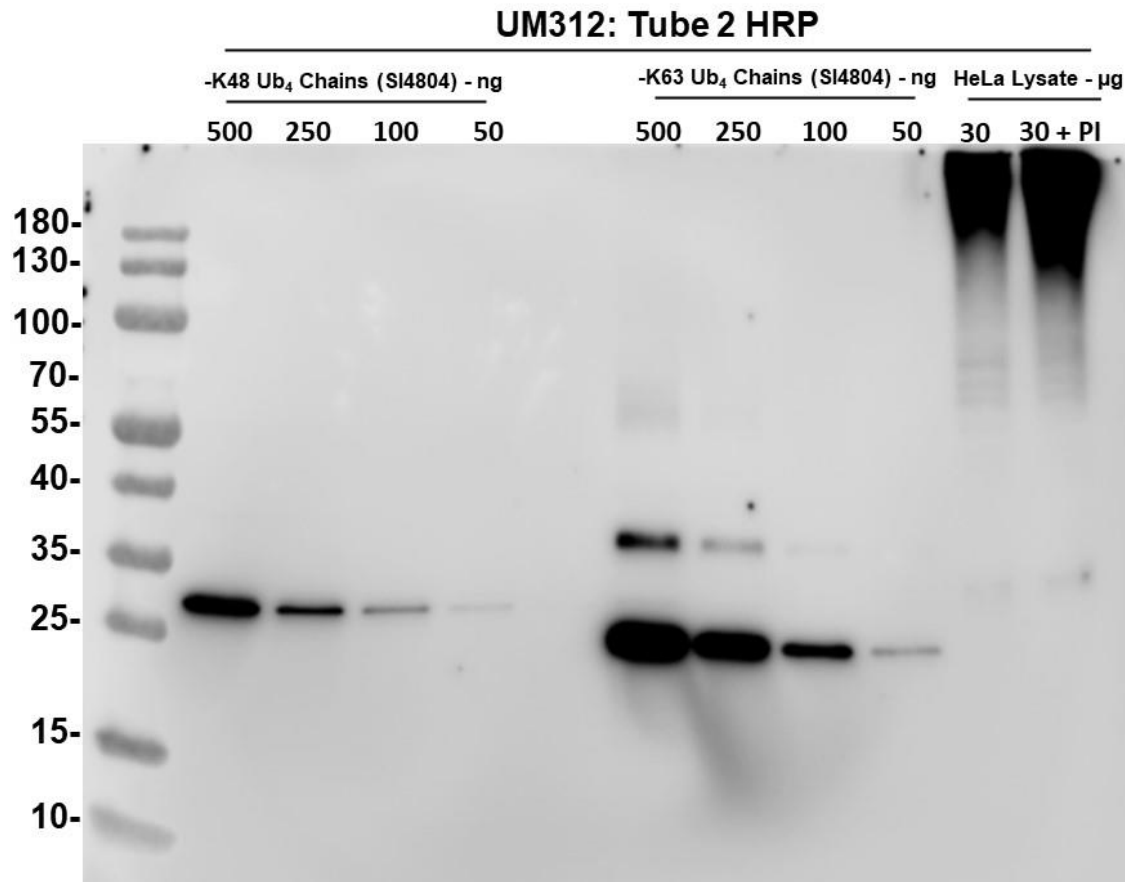
TUBEs conjugated with HRP allow for the detection of polyubiquitin and polyubiquitylated proteins by ligand blotting ("far Western") without heating the membrane. This reagent is a superior alternative to traditional polyubiquitin immunodetection techniques, such as anti-ubiquitin IgGs.

- Application:**
- Detection of polyubiquitylated proteins by ligand blotting
  - Pull down of polyubiquitylated proteins from cell lines, tissues and organs using a variety of readily available avidin supports
  - *In situ* labeling for detection of polyubiquitin by histochemistry

### Product Information

<b>Purity:</b>	>95% by SDS-PAGE
<b>Molecular Weight:</b>	38.5 kDa + HRP
<b>Tag:</b>	HRP
<b>Physical State:</b>	Liquid
<b>Quantity:</b>	50 µg
<b>Storage:</b>	-80° C. Avoid repeated freeze/thaw cycles

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TUBEs conjugated with HRP allowed detection of poly-ubiquitination chains and poly-ubiquitinated proteins from HeLa lysates using far western. TUBE-HRP diluted to 1:4000 in 5% milk PBS-T was used to successfully detect -K48 & -K63 poly-ubiquitin chains loaded at different amounts (500, 250, 10, 50 ng loading per well). TUBE HRP was also able to successfully detect poly-ubiquitination signature from HeLa lysates loaded at 30 µg/well. As seen in the QC image cells treated with proteasome inhibitor (PI) demonstrated enhanced ubiquitination signature as expected when probed with 1:4000 of TUBE2 HRP.

## References

- Donaghy, Ryan, et al. "The BRISC deubiquitinating enzyme complex limits hematopoietic stem cell expansion by regulating JAK2 K63-ubiquitination." *Blood*, vol. 133, no. 14, 2019, pp. 1560-1571.
- Lv, Kaosheng, et al. "CBL family E3 ubiquitin ligases control JAK2 ubiquitination and stability in hematopoietic stem cells and myeloid malignancies." *Genes & Development*, vol. 31, 2017, pp. 1007-1031.

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