

Anti-Ub TUBE 2, Biotin

Cat. # UM302

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.

Biotin-TUBEs 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

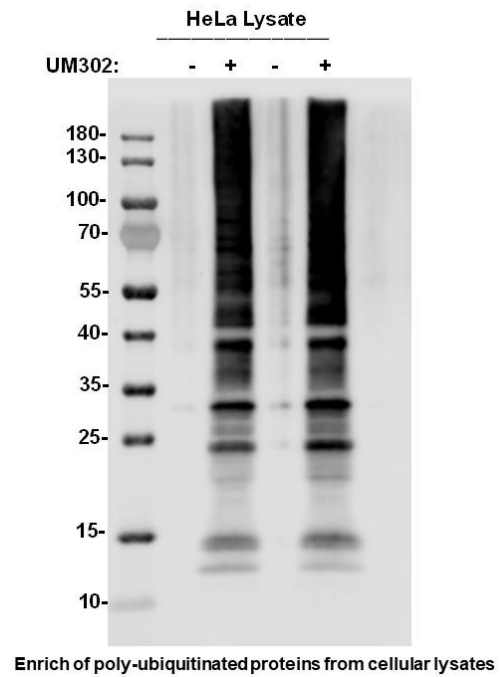
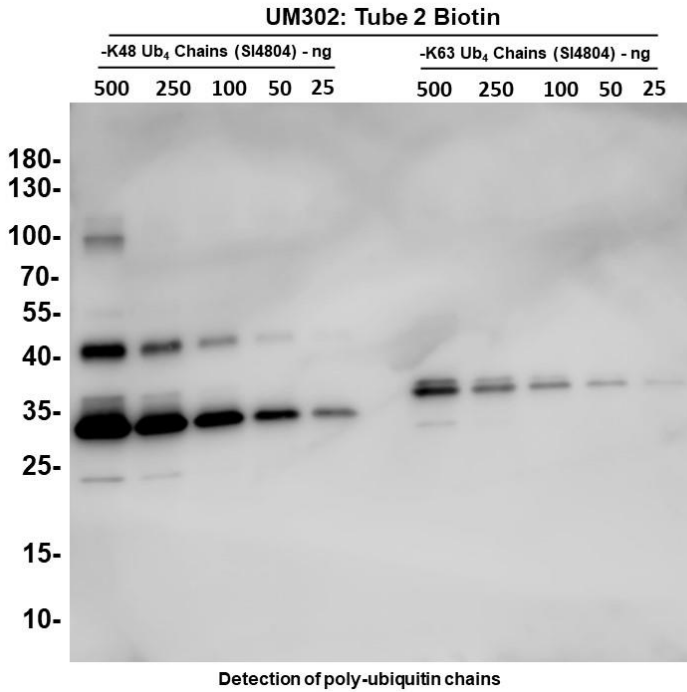
Product Information

Purity:	>95% by SDS-PAGE
Molecular Weight:	38.5 kDa + Biotin
Tag:	Biotin
Physical State:	Liquid
Quantity:	200 µg
Storage:	-80° C. Avoid repeated freeze/thaw cycles

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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TUBEs conjugated with Biotin allowed detection of poly-ubiquitination chains using far-western and enrichment of poly-ubiquitinated proteins from HeLa lysates. TUBE-Biotin diluted to 1:1000 in 3% BSA (PBS-T) was used to successfully detect -K48 & -K63 poly-ubiquitin chains loaded at different amounts (500, 250, 10, 50, 25 ng loading per well). TUBE2 Biotin was also able to successfully enrich poly-ubiquitinated proteins from 300 µg of HeLa lysates. -/+ indicates whether UM302 was added or not to cell lysates prior to enrichment using streptavidin magnetic beads. Characteristic poly-ubiquitination smears in '+' UM302 condition confirms successful enrichment.

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