TUBE 2 (Biotin)

Cat. # UM302

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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 ubiquitinated 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 polyubiquitinated state.

Biotin-TUBEs allow for the detection of polyubiquitin and polyubiquitinated 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.

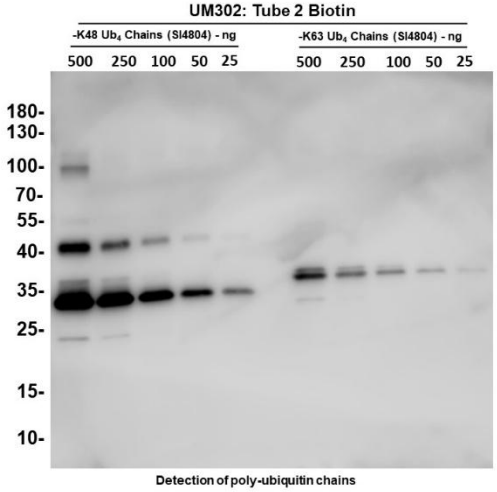
Application(s)

- Detection of poly-ubiquitinated proteins by ligand blotting
- Pull down of poly-ubiquitinated proteins from cell lines, tissues, and organs using a variety of readily available avidin supports
- In situ labeling for detection of poly-ubiquitin by histochemistry

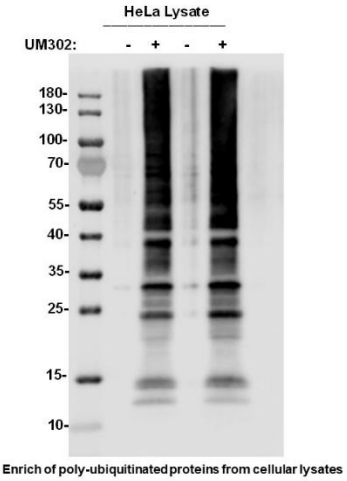
Product Specifications

Tag	Biotin
Purity	≥ 95% by SDS-PAGE
Molecular Weight	38.5 kDa + Biotin
Quantity	200 µg
Expression System	*E. coli*
Physical State	Liquid
Buffer	PBS
Concentration	Variable, depending on lot number
Stability & Storage	Over 1 year at -80°C. Avoid repeated freeze/thaw cycles

Product QC



Detection of poly-ubiquitin chains



Enrich of poly-ubiquitinated proteins from cellular lysates

TUBEs conjugated with biotin enabled the detection of polyubiquitin chains using far-western blotting and the enrichment of polyubiquitinated proteins from HeLa lysates. TUBE-Biotin, diluted 1:1000 in 3% BSA (PBS-T), was successfully used to detect K48- and K63-linked polyubiquitin chains at various loading amounts (500, 250, 100, 50, and 25 ng per well). TUBE2-Biotin also effectively enriched polyubiquitinated proteins from 300 µg of HeLa lysates. The symbols -/+ indicate whether UM302 was added to the cell lysates prior to enrichment using streptavidin magnetic beads. The characteristic polyubiquitination smear observed in the '+' UM302 condition confirms successful enrichment.

References

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4. Lopitz-Otsoa., et al., J Proteomics, 2012; 75, 2998-3014.
5. Kadimisetty K., et al., Methods Mol Biol, 2021;2365:185-202.