

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.

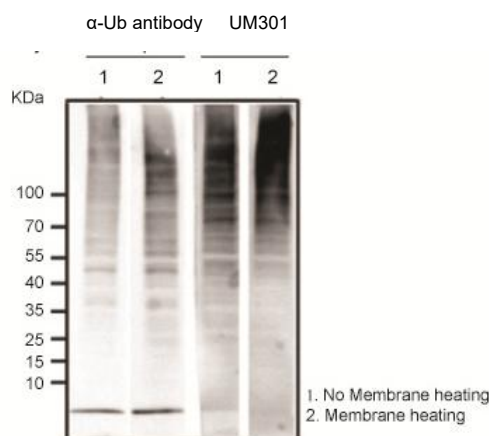
Biotin-TUBEs allow for the detection of polyubiquitin and polyubiquitinated proteins by ligand blotting (“far Western”) without heating the membrane. This reagent is a superior alternative to traditional polyubiquitin immunodetection techniques, such as anti-ubiquitin IgGs.

Application

- Detection of polyubiquitinated proteins by ligand blotting
- Pull down of polyubiquitinated 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 Specifications

Affinity tag	Biotin
Purity	≥ 95% by SDS-PAGE
Molecular Weight	38 kDa + Biotin
Quantity	200 µg
Expression System	<i>E. Coli</i>
Physical State	Liquid
Buffer	50 mM HEPES (pH 7.5), 150 mM NaCl, 10% glycerol
Solubility	> 30 mg/ml
Concentration	Variable, depending on lot number
Stability & Storage	Over 1 year at -80°C. Avoid repeated freeze/thaw cycles

Product QC

Approximately 40 µg of protein from Neuro 2A cells was subjected to SDS-PAGE, followed by electrophoretic transfer. Probing with α-ubiquitin was performed both with and without pre-treatment of the membrane by heat. Similarly, cellular lysates were probed with biotinylated TUBE1 at a 1:1000 dilution, with and without prior heating of the membrane. As shown in the image, UM301 outperformed the leading α-ubiquitin antibody in detecting polyubiquitination, both with and without membrane heating.

References

1. Garadi Suresh H et al., Mol Cell, 2024;84(12):2337-2352
2. Chen X., et al., Cell, 2023;186 (18):3903-3920.e21.
3. Reynolds SD., et al., JCI Insight, 2022;7(15): e157380.

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