MANUAL

Biotinylated TUBE 1 and TUBE 2

Catalog Numbers:
UM301 and UM302

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BACKGROUND

Ubiquitin and Polyubiquitylation

Ubiquitin is a small polypeptide that can be conjugated via its C-terminus to amine groups of lysine residues on target proteins. This conjugation is referred to as monoubiquitylation. Additional ubiquitin moieties can be conjugated to this initial ubiquitin utilizing any one of the seven lysine residues present in ubiquitin. The formation of these ubiquitin chains is referred to as polyubiquitylation. The two most well characterized forms of polyubiquitylation occur via linkage at lysine 48 (K48) or lysine 63 (K63). The most prevalent consequence of polyubiquitylation is the proteasome-mediated degradation of the target protein. Polyubiqiutination is a reversible process, however, as these chains are degraded and/or removed by proteases known as deubiquitylases (DUBs). The dynamic nature of this signaling represents a major obstacle to the isolation and functional characterization of polyubiquitylated proteins. For this reason, the ubiquitylation state of many proteins is unknown or poorly characterized.

TUBEs: A Revolution in Polyubiquitin Isolation and Characterization

Traditional strategies for characterization of ubiquitylated proteins often require immunoprecipitation of overexpressed ubiquitin with an epitope tag or the use of ubiquitin antibodies (expensive for large scale studies). Alternatively, isolation of polyubiquitylated proteins can be achieved with certain ubiquitin binding associated domains (UBAs), but these proteins display a low affinity for ubiquitin. Additionally, these strategies require the inclusion of inhibitors of both DUB and proteasome activity to protect the integrity of polyubiquitylated proteins. These conditions could alter cell physiology, which in turn may negatively impact the result or introduce experimental artifact. Tandem Ubiquitin Binding Entities (TUBEs) have been developed to overcome these problems and are licensed by LifeSensors, Inc. from Dr. Manuel Rodriquez at CIC bioGUNE. TUBEs are essentially tandem UBAs with dissociation constants for tetra-ubiquitin in the nanomolar range. TUBEs have also been demonstrated to protect proteins from both deubiquitylation and proteasome-mediated degradation, even in the absence of inhibitors normally required to block such activity. The nanomolar affinity of TUBEs for polyubiquitylated proteins allows for high efficiency in isolation and characterization of these proteins from cell lines, tissues and organs. **TUBE 1** displays an approximately 10-fold higher affinity for K63 tetra-ubiquitin relative to K48 linked species, and may allow for more efficient enrichment of proteins that are polyubiquitylated through this linkage type. Both **TUBE 2** and **TUBE 3** display equivalent affinities for both K63 and K48 tetra-ubiquitin, and are appropriate choices if the nature of the ubiquitin linkage is unknown.
The superior nature of TUBEs allows for efficient detection of polyubiquitylated proteins in their native state, while the versatility of TUBEs meets a wide range of experimental needs.

Biotin-TUBEs replace anti-ubiquitin antibodies, as well as the required blot heating, for the detection of polyubiquitylated proteins by ligand blotting ("far Western").

Other TUBEs Products:

Affinity tagged TUBEs allow for identification and characterization of polyubiquitin proteins by Western blotting, as well as isolation of proteins for downstream proteomic studies.

APPLICATIONS

Far Western blot analysis: Replace anti-Ubiquitin antibodies for the detection of polyubiquitylated proteins

BENEFITS

1. Up to 1000-fold higher affinity for polyubiquitin compared to single UBA form
2. Biotin TUBEs have higher specificity and affinity than antibodies

COMPONENTS

1. **TUBE 1, TUBE 2**

   Size: 1 x 200 µg (5mg/ml)

Buffer: 50 mM HEPES (pH 7.5), 150 mM NaCl, 10% glycerol

Storage: -80°C, avoid cycles of freezing and thawing

Please note that some physical characteristics and protocols are item specific. Please refer to individual product sheets or application notes now available at www.lifesensors.com for further information.

ADDITIONAL ITEMS REQUIRED

1. **Cell Lysis buffer:** RIPA buffer or other generic Tris-based buffers, pH 7.0-7.6 of choice could be used to generate cell or tissue lysates.

SUGGESTED PROTOCOL

1. Prepare cell extract for Western blot analysis using the extraction buffer of choice in the presence of protease inhibitors. To prevent deubiquitylation of proteins by DUBs during the extraction, we suggest using DUB inhibitor PR-619 (LifeSensors, cat. No.SI9619).
2. Clarify cell lysate by centrifugation for 10 min at 4°.
3. Prepare samples for SDS-PAGE using reducing SDS sample buffer. Load 30-50 μg of total protein per lane. The amount of protein for gel loading should be determined empirically.
4. Transfer to PVDF membrane according to manufacturer recommendations.
5. Block membrane in PBST containing 3%BSA (Cohn fraction V) for 1 h at room temperature (RT).
6. Incubate the membrane with Biotin-TUBE-1 or -2 (dilution 0.2-1.0 μg/ml in 3%BSA) for 1h at RT.
7. Wash the membrane with PBST 3 X 10 min.
8. Incubate the membrane with streptavidin conjugated HRP for 1h at RT. Manufacturer and dilutions should be determined empirically.
9. Wash the membrane with PBST at least 4 times, 10 min each prior to the detection of immunopositive protein bands using enhanced chemiluminescence reagent kit (ECL).

REFERENCES

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