Magnetic Beads-TUBE2 (High Capacity)

Cat. # UM502M

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 polyubiquitin state. UM502M was designed by coating high-capacity magnetic beads to allow superior enrichment of poly-ubiquitinated proteins along with minimizing non-specific binding to proteins in tissue and cellular lysates.

The affinity of TUBE 2 for K63 linked tetra-ubiquitin is approximately equal to K48 linked tetra-ubiquitin (5-10nM).

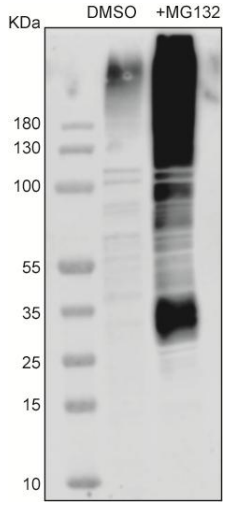
Application(s)

- Pull down of poly-ubiquitinated proteins from cell lines, tissues, and organs
- Protection of polyubiquitinated proteins from both deubiquitination and proteasomal degradation
- Bead-based ELISA for studying ubiquitinated proteome
- Label-free mass spectrometry for ubiquitinated proteomic analysis

Product Specifications

Affinity Tag	None
Purity	(prior to coupling) > 95% estimated by SDS-PAGE
Quantity	1 ml
Expression System	*E. coli*
Physical State	Liquid
Storage	+4°C. Avoid storage at lower temperatures

Product QC



Enrichment of total ubiquitinated proteome using UM502M. 100 µl of UM502M beads were added to 300 µg of cell lysates derived from HeLa cells treated with either DMSO or 1 µM MG-132 for 4 hours. The data represent overnight enrichment of both DMSO- and MG-132–treated lysates using UM502M at 4 °C on an end-to-end rotator. A characteristic increase in high-molecular-weight ubiquitin smears was observed in the MG-132–treated samples, indicating robust pull-down of the polyubiquitinated proteome. The enriched beads were resuspended in 30 µL of 1X Laemmli sample buffer and loaded onto a 10% SDS-PAGE gel. The western blot membrane was probed using anti-ubiquitin (VU101).

References

1. Garadi Suresh H et al., Mol Cell, 2024;84(12):2337-2352.
2. Kadimisetty K., et al., Methods Mol Biol, 2021;2365:185-202.
3. Hjerpe, R, et al., EMBO Rep., 2009; 10,1250-1258.