

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 polyubiquitin 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.

TMR-TUBE2 has a single TAMRA fluorophore (Exc. = 540 nm, Emm. = 578 nm) attached to the fusion tag of His6-TUBE2 (Cat. No. UM202). Since the fluorophore is attached to the tag, it does not affect the binding of the tandem ubiquitin binding domains to polyubiquitin chains.

Application(s)

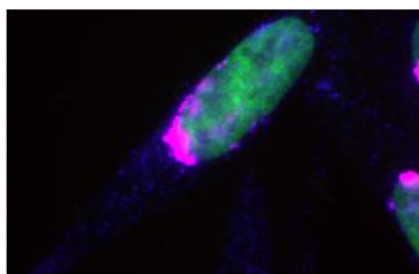
Cytochemical and histochemical staining of polyubiquitinated proteins for fluorescence microscopy.

Optimal conditions should be determined by the end user; suggested guidelines are as follows:

- Treatment of cells with proteasome inhibitors such as MG-132 to allow accumulation
- 5–20 minutes fixation with formaldehyde (2–4%), followed by a PBS wash
- 10-minute treatment with 0.5% Triton
- 1-hour incubation with TMR-TUBEs (1:500 dilution recommended as a starting point)

Product Specifications

Tag	His ₆
Purity	≥ 95% by RP-HPLC
Quantity	50 µg
Expression System	<i>E. coli</i>
Molecular Weight	39,078 Da
Physical State	Liquid
Buffer	PBS with 10% glycerol
Concentration	Variable, depending on lot number
Stability & Storage	Over 1 year at -80°C. Avoid repeated freeze/thaw cycles
Excitation/Emission Wavelengths	544/572 nm

Product QC**Cytochemical labeling of C2C12 mouse cells with TMR-TUBE2.**

Red: TMR-TUBE2; Blue: anti-ubiquitin monoclonal antibody (MAb); Green: DAPI (nuclear stain).

Image courtesy of V. Raz, Leiden University Medical Center.

References

1. Kadimisetty K., et al., Methods Mol Biol, 2021;2365:185-202.
2. Aillet, F., et al., Meth Mol Biol, 2012. 832: p. 173-183.
3. Hjerpe R., et al., EMBO Rep. 2009;10(11):1250-8.

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