FLR-TUBE2

Cat. # UM502F

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 ubiquitylated 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 polyubiquitylated proteins, allowing for detection at relatively low abundance. These properties effectively "capture" protein in its polyubiquitinated state.

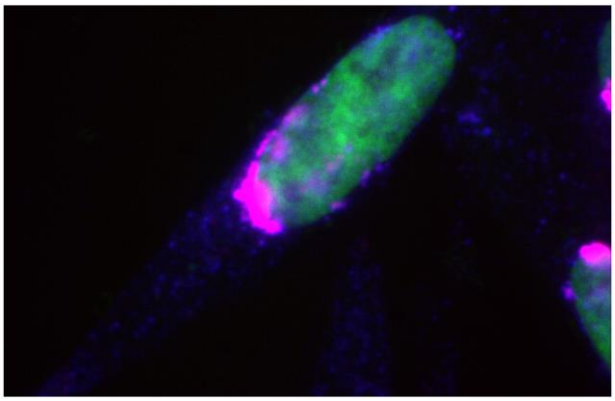
FLR-TUBE2 has an average of three fluorescein moieties (Exc. = 490 nm, Emm. = 520 nm) attached to the fusion tag of His_6-TUBE2 (Cat. No. UM202). Since the fluorophore is attached to the tag, it does not affect binding of the tandem ubiquitin binding domains to polyubiquitin chains.

Application:

- Cyto-/histo- chemical staining of polyubiquitylated proteins for fluorescence microscopy

Product Information

Purity:	$\geq$ 95% by RP-HPLC
Molecular Weight:	Average 39,953 Da
Physical State:	Liquid, PBS with 10% glycerol
Quantity:	50 μg at X mg/mL (depending on Lot No.)
Solubility:	Not determined
Storage:	-80° C. Avoid repeated freeze/thaw cycles



C2C12 mouse cells (Red: TMR-TUBE 2, Blue: anti-Ub mAb, FK2, Green: DAPI (nuclear)). Image provided by V. Raz, Leiden University Medical Center

General staining procedure (optimal conditions will need to be empirically determined by the end-user):

- Detectable signal may require treatment of cells with a proteasome inhibitor such as MG-132 (Cat.#SI9710) to allow accumulation of ubiquitylated proteins
- 5-20 minute fixation with formaldehyde (2-4%)
- PBS wash
- 10 minute treatment with 0.5% Triton
- 1 hour incubation with FLR-TUBEs (1:500 dilution as a recommended starting point)

References

Hjerpe, R, Aillet, F, Lopitz-Otsoa, F, Lang, V, England, P, and Rodriguez, MS., Efficient protection and isolation of ubiquitylated proteins using tandem ubiquitin-binding entities. *EMBO Rep.* **10,**1250-1258 (2009).