TUBE 2 -Alexa Fluor® 647

Cat. # **UM702A**

LifeSensors www.lifesensors.com

Background

LifeSensors has expanded the polyubiquitin technology with the development of TUBE (Tandem Ubiquitin Binding Entity) 2 site-specifically labeled with Alexa Fluor® 647. TUBE 2 -Alexa Fluor® 647 binds polyubiquitin chains with pan selectivity and provides a sensitive and cost-effective tool for determining the abundance of polyubiquitin in the cell, tissue extracts and *in vitro* ubiquitination/E3 ligase reactions. TUBEs have 100 to 1000-fold higher affinity for polyubiquitin chains compared to monomer ubiquitin binding domains (UBDs). TUBEs both stabilize and bind to ubiquitylated proteins, thereby effectively "capturing" proteins in their polyubiquitylated state [1-5].

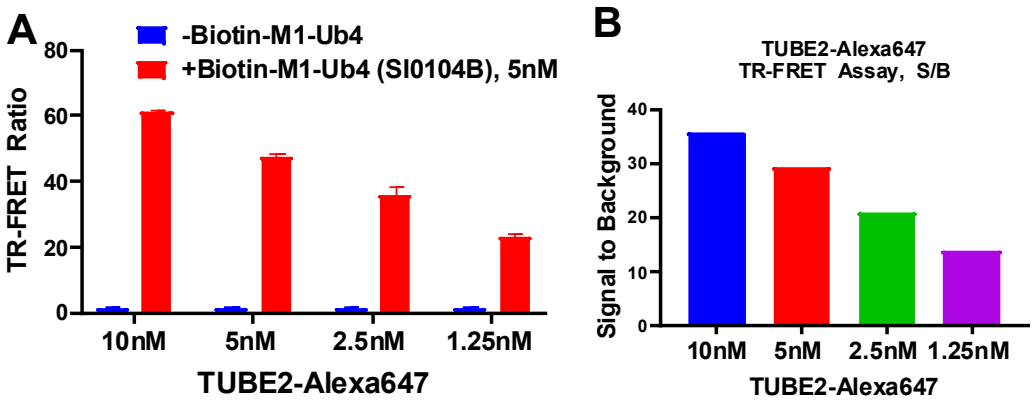
Applications

- In vitro E3 Ligase and substrate ubiquitination assay (polyubiquitination sensor).
- TR-FRET High Throughput Screening (HTS) assay
- Discovery of PROTAC and Molecular Glues in drug screening.
- Western blotting detection
- Immunohistochemistry staining

Product Specifications

Affinity Tag	His
Purity	≥ 95% by RP-HPLC and SDS-PAGE
Molecular Weight	39,494 Da
Quantity	25 µg
Expression System	*E. Coli*
Physical State	Liquid
Buffer	Phosphate Buffered Saline (PBS), 5% Glycerol
Concentration	Variable, dependent on lot number
Stability & Storage	Over 1 year at -80℃. Avoid repeated freeze/thaw cycles.

Product QC: TR-FRET Assay Application



Polyubiquitin Chain binding TR-FRET Assay: 5nM of Biotinylated-M1 (linear)-tetra ubiquitin (LifeSensors Cat#SI10104B) was incubated with indicated concentrations of TUBE 2-Alexa Fluour® 647 (LifeSensors Cat#UM702A) and 1.0nM of Streptavidin-Europium (Columbia Biosciences, Cat# D17-2212) in a total volume of 10µl/well in 384-well plate. TR-FRET signal was measured using Clariostar (BMG LabTech) plate reader. **Figure A** shows the TR-FRET ratio [Alexa647(665-10) / Europium(620-10)*100], and **Figure B** shows the Signal to Background (n=4).