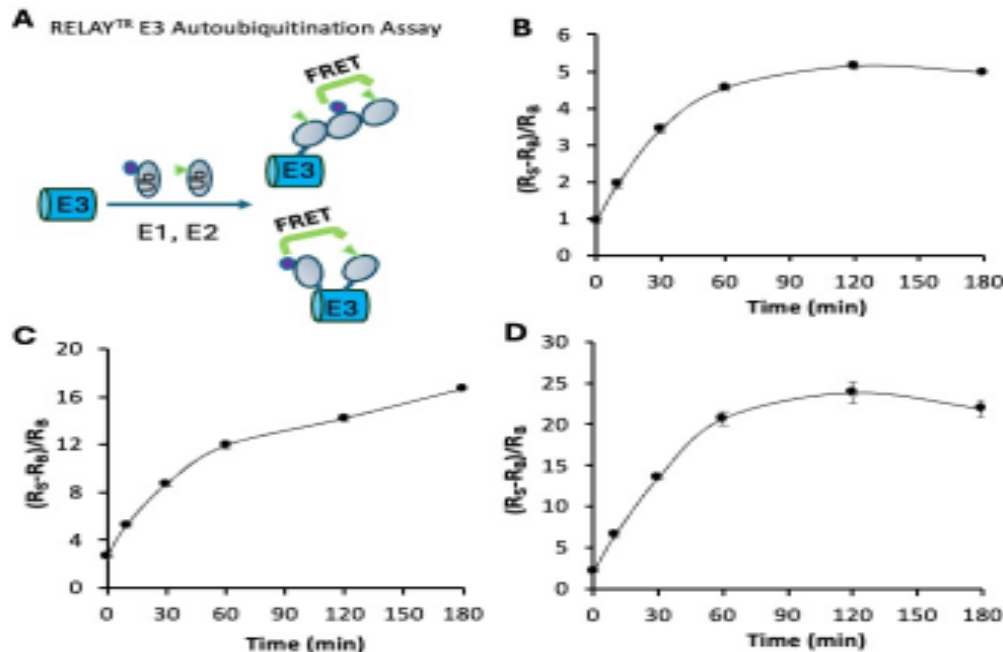


100X RELAY^{TR} Terbium/Fluorescein Ubiquitin Mix

Catalog # T2501-200, T2501-2K, T2501-5K

Also Known as:	Terbium-Ubiquitin, Fluorescein-Ubiquitin, Tb-Ub, Fluorescein Ub
Quantities:	200 Rxns for T2501; 2000 Rxns for T2501-2K; 5000 Rxns for T2501-5K
MW (no tag):	Tb-Ub (9.5 kDa), Fluorescein-Ub (9 kDa)
Species:	Human
Source:	Recombinant human ubiquitin
Tag:	No
Stock Buffer:	20 mM HEPES, pH 7.2, 50 mM NaCl, 2 mM TCEP
Concentration:	See tube label
Quality Assurance:	>95% purity by SDS-PAGE; Validated by RELAY ^{TR} FRET ubiquitination assays.

Image



A. Schematic of RELAY^{TR} FRET assay monitoring E3 autoubiquitination.

B. Autoubiquitination of 25 nM CHIP detected by RELAY^{TR} FRET assays with Tb-Ub and Fluorescein-Ub. The ratios (R) of fluorescence intensity at 520 nm and 490 nm from reactions containing E1/E2/E3/Ub Mix/ATP and E1/E2/Ub Mix/ATP were Signal (R_S) and Blank (R_B), respectively. The signal-to-blank ratio was calculated by using the formula of $(R_S - R_B)/R_B$. Time 0 is ~5 min after ATP addition.

C. Autoubiquitination of 50 nM MDM2 detected by RELAY^{TR} FRET assays.

D. Autoubiquitination of 12.5 nM MURF2 detected by RELAY^{TR} FRET assays.

Description: Time resolved (TR)-FRET offers a sensitive, low background, stable and homogenous assay for determination of protein ubiquitination. Terbium-ubiquitin and fluorescein-ubiquitin are a pair of modified ubiquitin suitable for TR-FRET assays to assess formation of polyubiquitin chains or multi-monoubiquitination, thereby reporting the activity of E2 and E3 enzymes in the reaction. The concentration and molar ratio of terbium-ubiquitin and fluorescein-ubiquitin in the RELAY^{TR} Terbium/Fluorescein Ubiquitin Mix are optimized to achieve excellent signal-to-blank ratio and validated by multiple E3 autoubiquitination assays. The blank reaction contains UbE1, E2, Terbium/Fluorescein Ubiquitin Mix, and ATP.

Typical protein concentration ranges are: UbE1 (10-30 nM), E2 (50-250 nM) and E3 (10-250 nM). Reaction time is usually 1-3 hours in kinetic or end point assay.

Storage: Store at -80°C; Avoid multiple freeze-thaw cycles.

Notes:

- 1) The Terbium-ubiquitin and Fluorescein-ubiquitin Mix is tested in multiple ubiquitin ligase autoubiquitination assays and generated desirable signal to blank ratios. It may not be optimal for certain ubiquitin ligases.
- 2) A TR-FRET capable plate reader is required. Our assays were performed using a PHERAstar FS instrument with the 337/520/490 nm filter set. Intergration started at 50 µs, and the intergration time was 400 µs.

Literature: <https://www.bmglabtech.com/en/tr-fret/>