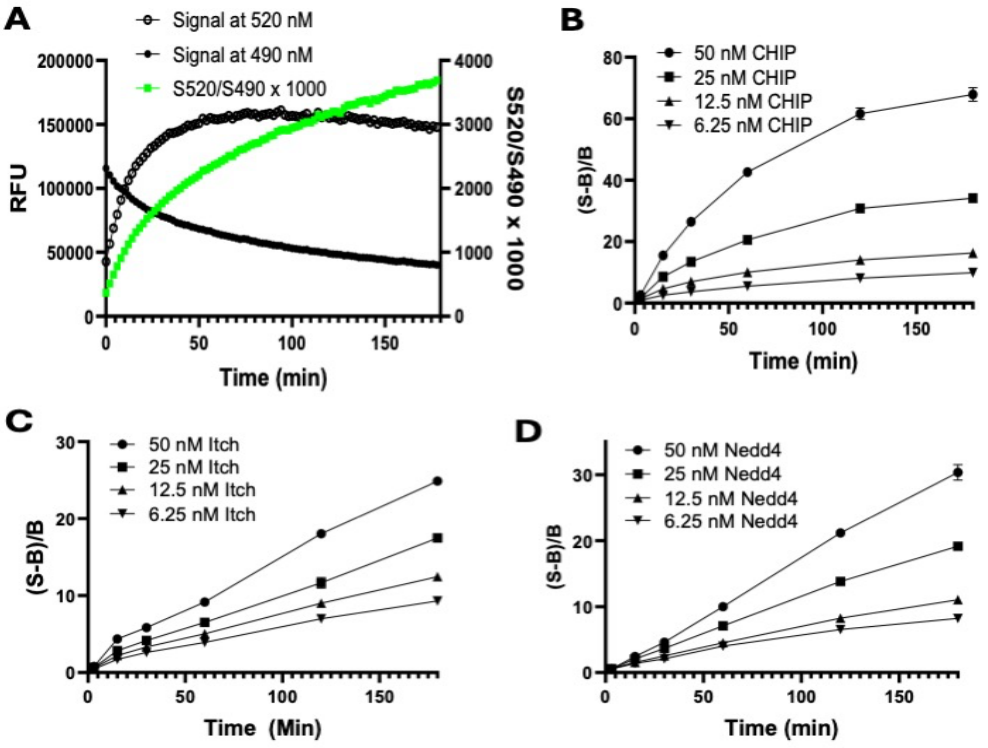
100X RELAY^{TR} Terbium/Fluorescein Ubiquitin Mix

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

Also Known as:	Terbium-Ubiquitin, Fluorescein-Ubiquitin, Tb-Ub, Fluorescine 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. Kinetic mode monitoring 25 nM CHIP autoubiquitination. RELAY^{TR} Terbium/Fluorescein Ubiquitin Mix (Catalog# T2501) was used in all reactions.

B. CHIP concentration-dependent autoubiquitination monitored by RELAY^{TR} FRET assays with Tb-Ub and Fluorescein-Ub. S520/S490 ratios from reactions with ATP were positive Signal (S), and without ATP were Backgrounds (B). The signal-to-background ratio was calculated by using the formula of (S-B)/B.

C. Itch concentration-dependent autoubiquitination, similar to B.

D. Nedd4 concentration-dependent autoubiquitination, similar to B.

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, 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-background ratio as validated in various polyubiquitination assays. Optimization of protein concentrations are often necessary to achieve a desirable signal-to-background ratio. Typical protein concentration ranges are: UbE1 (10-30 nM), E2 (25-250 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 provided Terbium-ubiquitin and Fluorescein-ubiquitin Mix may not be optimal for certain protein ubiquitination. Users may order individual reagents to optimize their ubiquitination reaction.
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 intergration time was 400 µs.

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