100X RELAY^{TR} Europium/Cy5 Ubiquitin Mix

Catalog # T3501-200, T3501-2K, T3501-5K

Also Known as: Europium-Ubiquitin, Cy5-Ubiquitin, Eu-Ub, Europium-Ub, Cy5-Ub

NCBI Reference: 200 Rxns for T3501-200; 2000 Rxns for T3501-2K; 5000 Rxns for T3501-5K

MW (no tag): Eu-Ub (9.5 kDa); Cy5-Ub (9.1 kDa)

Species: Human

Source: Recombinant human ubiquitin

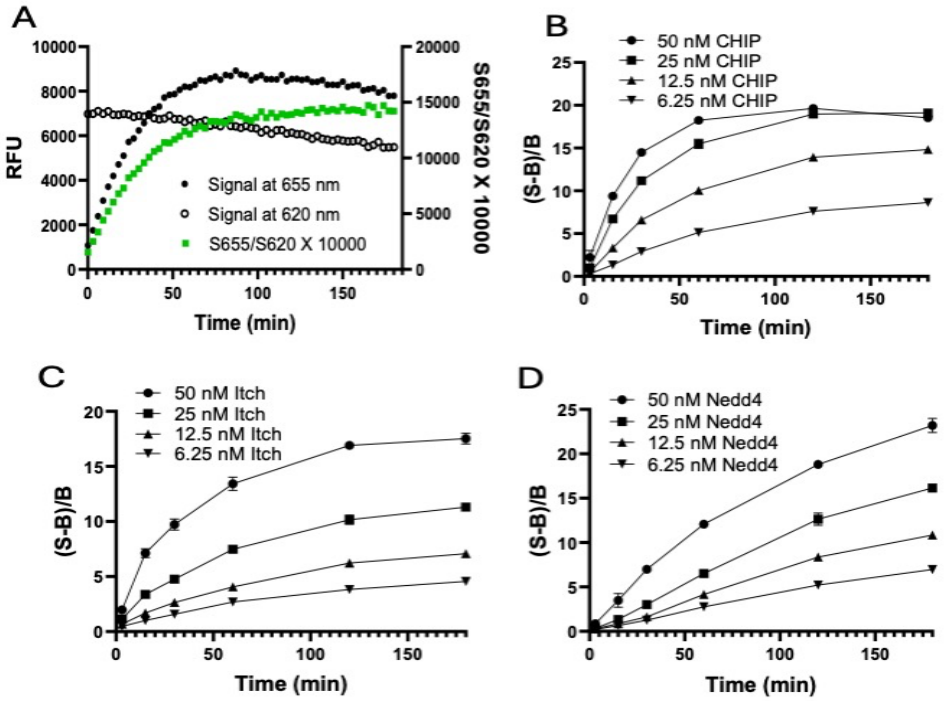
Tag: No

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} Europium/Cy5 Ubiquitin Mix (Catalog# T3501) was used in all reactions.

B. CHIP concentration-dependent autoubiquitination monitored by RELAY^{TR} FRET assays with Eu-Ub and Cy5-Ub. S655/S620 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. Europium-ubiquitin and Cy5-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 europium-ubiquitin and Cy5-ubiquitin in the RELAY^{TR} Europium/Cy5 Ubiquitin Mix are optimized to achieve excellent signal-to-background ratio in ubiquitination reactions. Optimization of protein concentrations is 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.
Note:	1) RELAY^{TR} Europium/Cy5 Ubiquitin Mix may not be optimal for certain protein ubiquitination. Users may order individual reagents to optmize their ubiquintion reaction. 2) A TR-FRET capable plate reader is required. Our assays were performed using a PHERAstar FS instrument with the 337/665/620 nm filter set. Intergration started at 50 µs, and intergration time was 400 µs.
Literature:	https://www.bmglabtech.com/en/tr-fret/