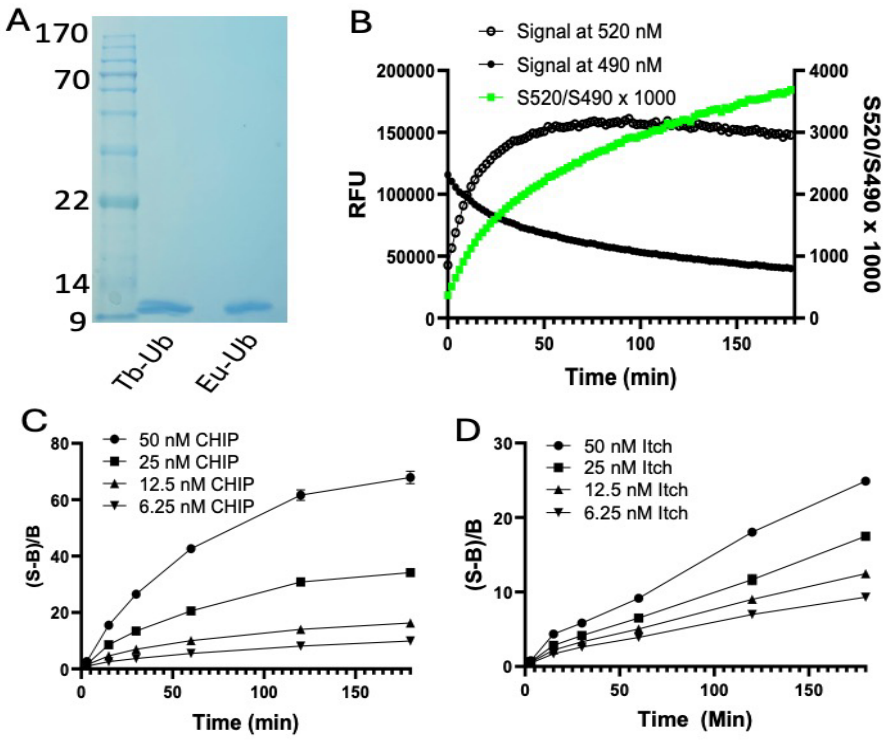
Terbium-Ubiquitin

Catalog # T2001-5, T2001-25, T2001-100

Also Known as:	Tb-Ub; Tb-Ubiquitin; Terbium-Ub
Quantities:	5 µg for T2001-5; 25 µg for T2001-25, 100 µg for T2001-100
MW (no tag):	9.5 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. Coomassie staind SDS-APAGE with 5 µg Tb-Ub or Eu-Ub.

B. Kinetic mode monitoring 25 nM CHIP autoubiquitination. RELAY^{TR} Terbium/Fluorescein Ubiquitin Mix (Catalog# T2501) was used in all reactions.

C. 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.

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

Description: A single terbium chelate is covalently labeled on recombinant human ubiquitin (Ub). All lysines, the N-terminal methionine and the C-terminal glycine of Ub are available for ubiquitination. Pairing with Fluorescein-Ub (Catalog # T2201), this product has been validated in TR-FRET assays to assess formation of K48 or K63 polyubiquitin chains and autoubiquitination of E3 Ub ligases, in which excellent signal-to-background ratios were achieved.

The assay condition should be optimized to achieve a desirable signal-to-background ratio, including reaction time and concentrations of UbE1, E2, E3, Terbium-Ub and Fluorescein-Ub. A typical range of Terbium-Ub concentration in TR-FRET assays is 10-30 nM, and Fluorescein-Ub concentration is usually 6-15 fold molar excess of Terbium-Ub. UbE1, E2 and E3 concentrations are usually at the range of 10-30 nM, 25-250 nM, 10-250 nM, respectively.

100X RELAY^{TR} Terbium/Fluorescein Ubiquitin Mix (Catalog # T2501) with optimized concentration and ratio of these two Ub moieties were used in all of our RELAY^{TR} FRET ubiquitination assays.

Reaction time is usually 1-3 hours in kinetic or end point assay.

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

Note: 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

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