Europium-Ubiquitin

Catalog # T3001-5, T3001-25, T3001-100

Also Known as: Eu-Ub; Eu-Ubiquitin; Europium-Ub

QUantities: 5 µg for T3001-5; 25 µg for T3001-25; 100 µg for T3001-100

MW (no tag): 9.5 kDa

Species: Human

Source: Recombinant human ubiquitin

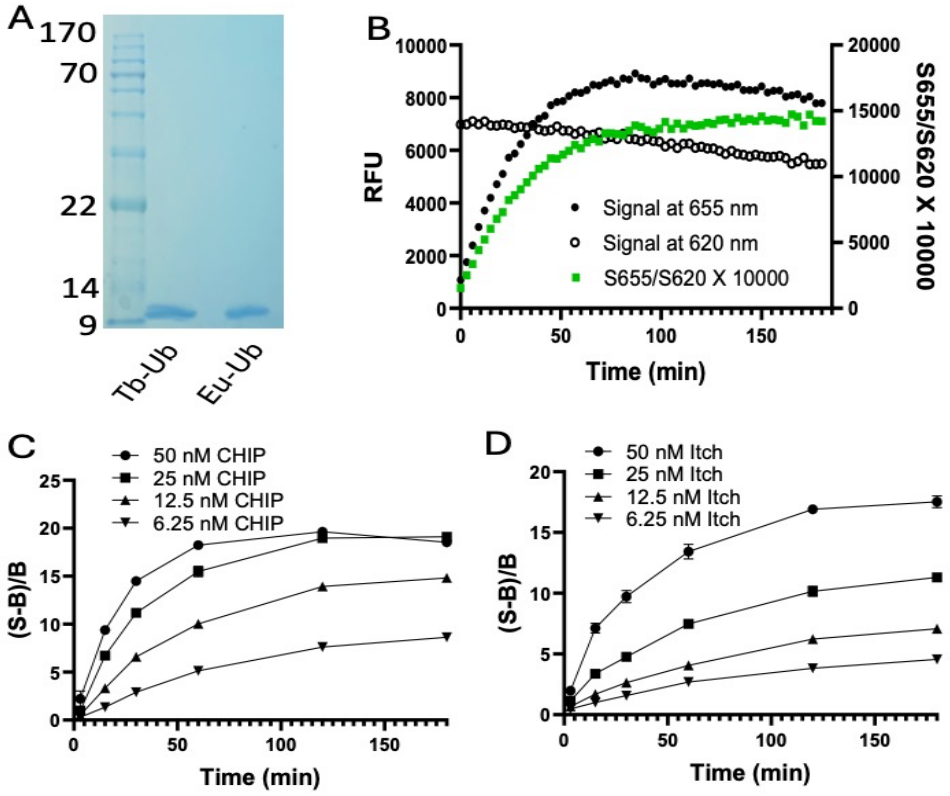
Tag: No

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

C. 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.

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

Description: A single europium 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 Cy5-Ub (Catalog # T3201), this product has been validated in RELAY^{TR} FRET assays to assess autoubiquitination of E3 Ub ligases, in which excellent signal-to-background ratios were achieved.

The RELAY^{TR} FRET ubiquitination assay should be optimized to achieve a desirable signal-to-background ratio, including reaction time and concentrations of UbE1, E2, E3, Europium-Ub and Cy5-Ub, A typical range of europium-Ub concentration is 25-100 nM, and Cy5-Ub concentration is usually 0.5-2X of europium-Ub. UbE1, E2 and E3 concentrations are usually at the range of 10-30 nM, 25-250 nM, and 10-250 nM, respectively.

100X RELAY^{TR} Europium/Cy5 Ubiquitin Mix (Catalog # T3501) with optimized concentration and ratio of these two Ub moieties were used in our E3 autoubiquitination 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 337/665/620 nm filter set. Intergration started at 50 µs, and intergration time was 400 µs.

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