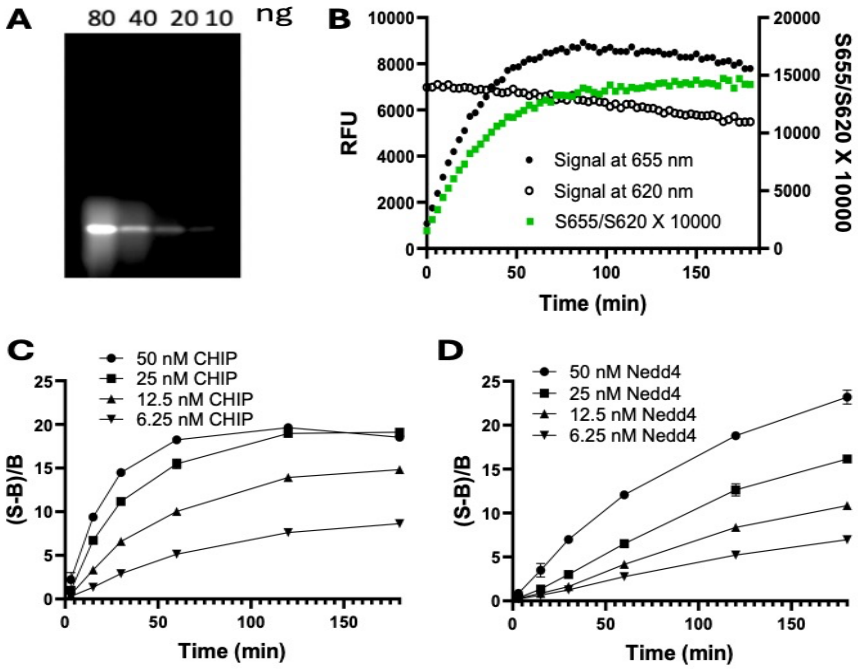
Cy5-Ubiquitin

Catalog # T3201-100; T3201-1000

Also Known as: Cy5-Ub; Cyanine 5 ubiquitin
Quantities: 100 µg for T3201-100; 1 mg for T3201-1000
MW (no tag): 9.1 kDa
Species: Human
Source: Human recombinant
Tag: No
Stock Buffer: 20 mM Tris, pH 7.6 at 4 °C, 150 mM NaCl, and 10% glycerol.
Concentration: See tube label
Quality Assurance: >90% by SDS-PAGE; Validated by RELAY^{TR} FRET E3 autoubiquitination assays.

Results:



A. Fluorescent visualization of Cy5-Ub separated on an SDS-PAGE.

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. Nedd4 concentration-dependent autoubiquitination, similar to C.

Description: A single Cy5 moiety is covalently labeled on recombinant human ubiquitin (Ub). All lysines, the N-terminal methionine and the C-terminal glycine of ubiquitin are available for ubiquitination. It pairs with Europium-Ubiquitin (Catalog# T3001) in TR-FRET assays assessing formation of polyubiquitin cbains (see Results section above).

The TR-FRET assay condition 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-ubiquitin concentration in TR-FRET assays is 25-100 nM, and Cy5-ubiquitin concentration is usually 0.5-2X of europium-ubiquitin. 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

Notes: 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/