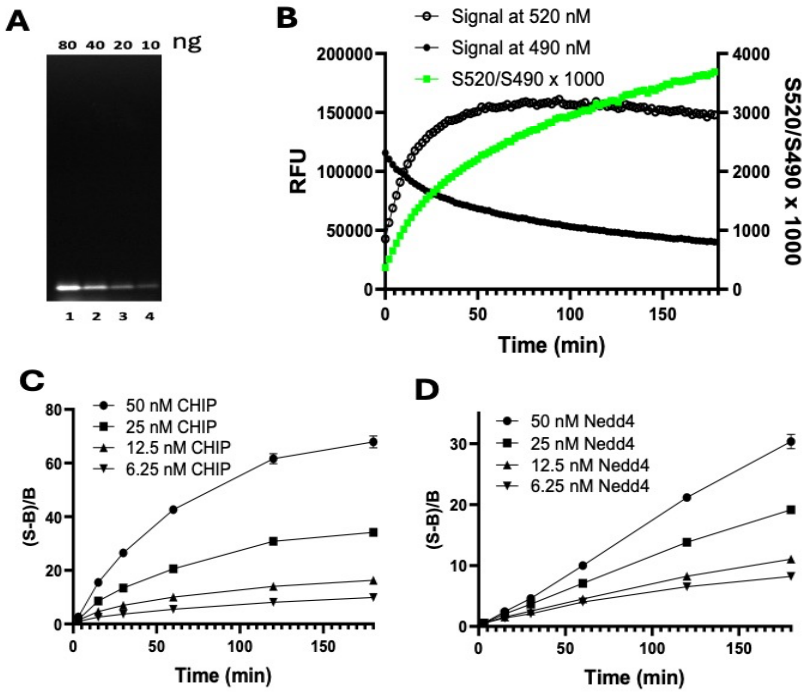
Fluorescein-Ubiquitin

Catalog # T2201-100, T2201-1000

Also Known as: Fluorescein-Ub; Fl-Ub
Quantities: 100 µg for T2201-100; 1 mg for T2201-1000
MW (no tag): 9 kDa
Species: Human
Source: Human recombinant protein
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: >95% by SDS-PAGE; Validated by TR-FRET ubiquitination assays.

Results:



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

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. Nedd4 concentration-dependent autoubiquitination, similar to C.

Description: A single fluorescein moiety is covalently labeled on recombinant human ubiquitin. The N-terminal methionine, the C-terminal glycine, and all lysine residues are available for ubiqiutination. It pairs with Terbium-Ubiquitin (Catalog# T2001) in TR-FRET assays to assess formation of polyubiquitin chains (see Results section above).

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, terbium-ubiquitin, and fluorescein-ubiquitin. A typical range of terbium-ubiquitin concentration in TR-FRET assays is 10-30 nM, and fluorescein-ubiquitin concentration is usually 6-15 fold molar excess of terbium-ubiquitin. 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/FluoresceinUbiquitin Mix (Catalog# T2501) with optimized concentration and ratio of these two Ub moieties were used in 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 50 µs, and intergration time was 400 µs.

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