

Sumo1, Sumo2, Sumo 3 Conjugation Kit

(Cat. # J3140)

*This kit is sufficient for setting up 25 x 20 μ l reactions.

Description

Sumo1, Sumo2, Sumo 3 Conjugation Kit is designed for assaying the conjugation of Sumo1, Sumo2 and Sumo3 to protein substrates *in vitro*. In a protein sumoylation reaction, a Sumo protein is first activated by the Sumo activating enzyme SAE1/SAE2 (E1) in an ATP hydrolysis-dependent manner; activated Sumo is then transferred to the Sumo conjugating enzyme Ube2I (E2) by formation of a thiolester bond with the catalytic cysteine residue of Ube2I; Sumo-charged Ube2I can mediate protein sumoylation in the presence of an appropriate Sumo ligase (E3).

A typical 20 μ l reaction contains 100 nM SAE1/SAE2, 2 μ M 6xHis-Ube2I, 50 μ M Sumo1 (or Sumo2, or Sumo3), 4 mM ATP, 2 μ M E3 (not supplied) and 2 μ M protein substrate (no supplied) in the reaction buffer.

Components

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|--------------------------------|-------------|
| • 20X SAE1/SAE2 (2 μ M) | 25 μ l |
| • 20X 6xHis-Ube2I (40 μ M) | 25 μ l |
| • 10X Sumo1 (500 μ M) | 50 μ l |
| • 10X Sumo2 (500 μ M) | 50 μ l |
| • 10X Sumo3 (500 μ M) | 50 μ l |
| • 10X ATP (40 mM) | 125 μ l |
| • 10X Sumoylation Buffer | 1 ml |

Note

1. 10X Reaction Buffer: 400 mM Tris, pH 7.1 at 37°C, 400 mM NaCl, 10 mM β ME, 50 mM $MgCl_2$

