

## Sumo3 Conjugation Kit (Cat. # J3130)

\* This kit is sufficient for setting up 25 x 20  $\mu$ l reactions.

### **Description**

Sumo3 Conjugation Kit is designed for assaying the conjugation of 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  $\mu$ l reaction contains 100 nM SAE1/SAE2, 2  $\mu$ M 6xHis-Ube2I, 50  $\mu$ M Sumo3, 4 mM ATP, 2 $\mu$ M E3 (not supplied) and 2  $\mu$ M protein substrate (no supplied) in the reaction buffer.

### **Components**

- |                                |             |
|--------------------------------|-------------|
| • 20X SAE1/SAE2 (2 $\mu$ M)    | 25 $\mu$ l  |
| • 20X 6xHis-Ube2I (40 $\mu$ M) | 25 $\mu$ l  |
| • 10X Sumo3 (500 $\mu$ M)      | 50 $\mu$ l  |
| • 10X ATP (40 mM)              | 60 $\mu$ l  |
| • 10X Reaction Buffer          | 100 $\mu$ l |

### **Note**

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

