

Nedd8 Conjugation Kit (Cat. # J3150)

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

Description

Nedd8 Conjugation Kit is designed for assaying the conjugation of Nedd8 to protein substrates *in vitro*. In a protein neddylation reaction, Nedd8 is first activated by the Nedd8 activating enzyme APPBP1/UBA3 (E1) in an ATP hydrolysis-dependent manner; activated Nedd8 is then transferred to the Nedd8 conjugating enzyme Ube2M (UbcH12) (E2) by formation of a thiolester bond with the catalytic cysteine residue of UbcH12I; Nedd8-charged Ube2M (UbcH12) can mediate protein neddylation in the presence of an appropriate Nedd8 ligase(E3).

A typical 20 µl reaction contains 100 nM APPBP1/6xHis-UBAs, 2 µM 6xHis-Ube2M (UbcH12), 15 µM 6xHis-Nedd8, 4 mM ATP, 2 µM E3 (not supplied) and 2 µM protein substrate (no supplied) in the Neddylation Buffer.

Components

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|------------------------------------|--------|
| • 20X APPBP1/6xHis-UBA3 (2 µM) | 25 µl |
| • 20X 6xHis-Ube2M (UbcH12) (40 µM) | 25 µl |
| • 10X 6xHis-Nedd8 (150 µM) | 50 µl |
| • 10X ATP (40 mM) | 60 µl |
| • 10X Neddylation Buffer | 100 µl |

Note

1. 10X Neddylation Buffer: 400 mM Tris, pH 7.1 at 37°C, 400 mM NaCl, 10 mM β-ME, 50 mM MgCl₂

