

MATERIAL DATA SHEET

NEDD8 Conjugation Initiation Kit

Cat. # K-800

This kit is designed for the conjugation of the ubiquitin-like modifier NEDD8 to protein substrates *in vitro*, which requires the activities of the heterodimeric human E1 activating enzyme (APPBP1/Uba3) and the UbcH12 E2 enzyme. The E1 enzyme charges the NEDD8 by forming an ATP-dependent high energy thiolester bond with the active site cysteine of Uba3. The activated NEDD8 is subsequently transferred to UbcH12 and this E2-S-Ub thiolester complex can be used for the conjugation of NEDD8 to protein substrates in the presence of the appropriate E3 enzymes (not supplied).

NOTE: Kit contains reagents sufficient for 10 x 20 µl reactions.

Product Information

	<u>Concentration</u>	<u>Volume</u>
Supplied:		
1. 10X NEDD8 E1 Enzyme	X mg/ml (X µM)	20 µl
2. 10X NEDD8	X mg/ml (X µM)	20 µl
3. 10X UbcH12	X mg/ml (X µM)	20 µl
4. 10X Mg-ATP Solution	X mM	20 µl
5. 10X Reaction Buffer	500 mM Hepes pH 8 500 mM NaCl	20 µl

Storage: Store at -80°C. Avoid multiple freeze/thaw cycles.

Background

The ubiquitin-like NEDD8 is conjugated to a variety of proteins in the presence of UbcH12 and an E1 activating enzyme. The NEDD8 E1 enzyme uses ATP to adenylate the C-terminal glycine residue of NEDD8, forming a high-energy thiolester bond. The second step is the trans-esterification reaction whereby the activated NEDD8 is transferred to the active site cysteine of UbcH12. UbcH12 is a member of the E2 family and is homologous to ubiquitin-conjugating enzymes, but is specific for the conjugation of NEDD8 to a variety of target proteins. Nedd8 plays a critical regulatory role in cell proliferation and development, and modifies nearly all members of the Cullin family.

Literature

- References:**
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