# abcam

## Product datasheet

## Neddylation Assay Kit ab139468

## 1 图像

#### 概述

产品名称 Neddylation Assay试剂盒

样品类型 Purified protein

检测类型 Direct

产品概述 Abcam Neddylation Assay Kit (ab139468) provides the means of generating a thioester-linked

NEDD8-conjugated E2 enzyme, utilizing the first two steps in the NEDD8 cascade, for use in the NEDDylation of E3 ligases and target substrate proteins (user supplied). A NEDD8-specific antibody is provided for detection of NEDDylated proteins via SDS-PAGE and western blotting.

This kit provides sufficient material for 20 x 20µL reactions.

说明

NEDD8 (neural precursor cell expressed developmentally downregulated gene 8) is an ubiquitin-like protein, with approximately 60% identity to ubiquitin (the highest homology of any ubiquitin-like protein). Conjugation of mature NEDD8 (via its exposed C-terminal glycine residue) to specific lysine residues on a limited number of cellular target proteins via isopeptide bonds, allows

NEDD8 to play a critical regulatory role in cell proliferation and development.

The NEDD8 conjugation pathway proceeds by a mechanism analogous to that of the ubiquitin conjugation cascade, consisting of a dedicated NEDD8 E1 activating enzyme (a heterodimeric complex consisting of a regulatory subunit APP-BP1 and a catalytic subunit Uba3), E2 conjugating enzyme (UbcH12) and substrate specific E3 ligases.

An increasing number of substrates for NEDDylation, including the tumor suppressors p53 and VHL, and EGFR have been identified in addition to members of the well-characterized cullin family of proteins, that play a structural role in ubiquitin E3 ligase complexes such as SCF.

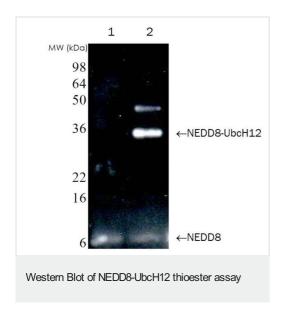
Cullin NEDDylation in SCF complexes is facilitated by the RING domain containing Rbx1 (Roc1) SCF subunit, which is thought to act as a NEDD8 E3 ligase, and enhances the complex's ubiquitin E3 ligase activity.

NEDDylation of p53 is mediated by its ubiquitin E3 ligase Mdm2, providing an additional control mechanism for inhibition of its transcriptional activity. E3 independent NEDDylation of p53 has also been demonstrated *in vitro*. Reconstitution of the NEDD8 conjugation pathway *in vitro* using recombinant proteins is readily achieved and has been used to investigate a number of NEDD8 modification systems.

#### 性能

Components	
20X Mg-ATP Solution	1 x 25µl
2X Non-reducing Gel Loading Buffer	1 x 400µl
10X NEDD8 Activating Enzyme Solution	1 x 40µl
20X UbcH12 Solution (E2)	1 x 20µl
10X NEDD8 Solution	1 x 40µl
NEDD8 Antibody Solution	1 x 25µl
10X NEDDylation Buffer	1 x 40µl

#### 图片



NEDD8 modified proteins were detected by Western Blotting using NEDD8 antibody. Results demonstrate formation of NEDD8-UbcH12 thioester in the presence of Mg-ATP (lane 2) but not when Mg-ATP is absent (lane1).

An additional higher band (approx. ~ 44kDa) is sometimes observed, possibly due to NEDD8-NEDD8 isopeptide linkage formation.

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