NEDD8-Rhodamine 110

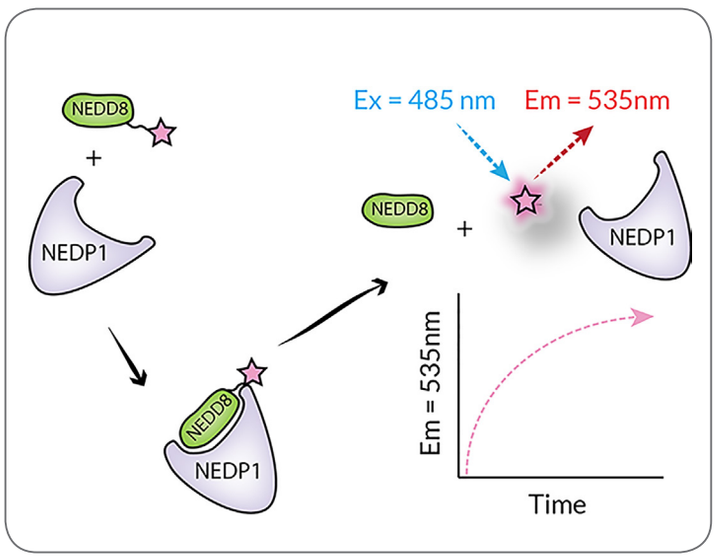
Cat. No. SBB-PS0003
Lot. No. 163060003

South Bay Bío

NEDD8 Rhodamine 110

NEDD8 (Neural Precursor Cell Expressed, Developmentally Down-Regulated 8) is a ubiquitin like protein that plays an important role in regulating development and the cell cycle. NEDD8 is conjugated to a target protein via a signaling cascade similar to ubiquitin: a NEDD8 specific E1 activating enzyme (APPBP1/UBA3) adenylates the c-terimnus of NEDD8 which is than subsequently passed to an E2 conjugating enzyme (UBE2M or UBE2F) that facilitates covalent attachment of NEDD8 to a cullin subunit of an SCF E3 ubiquitin ligase.

NEDD8-Rh 110 can be used as a substrate for enzymes exhibiting deNEDDylating activity, e.g UCH-L3, UCH-L1, COP9 Signalosome, and NEDP1. This product consists of a full-length, mature NEDD8 polypeptide (amino acids 1-76) recombinantly expressed in *E.coli*, conjugated on its c-terminus to a quenched Rhodamine110 dye. Once hydrolyzed the free rhodamine provides excellent utility for real time assessment of enzyme activity at excitation (485 nm) and emission (535 nm). Typical working range is 50-500nM.



Product Information

Quantity: 50µg **Molecular Weight:** 8.9 kDa

Concentration: 170 µM, 1.5 mg/mL

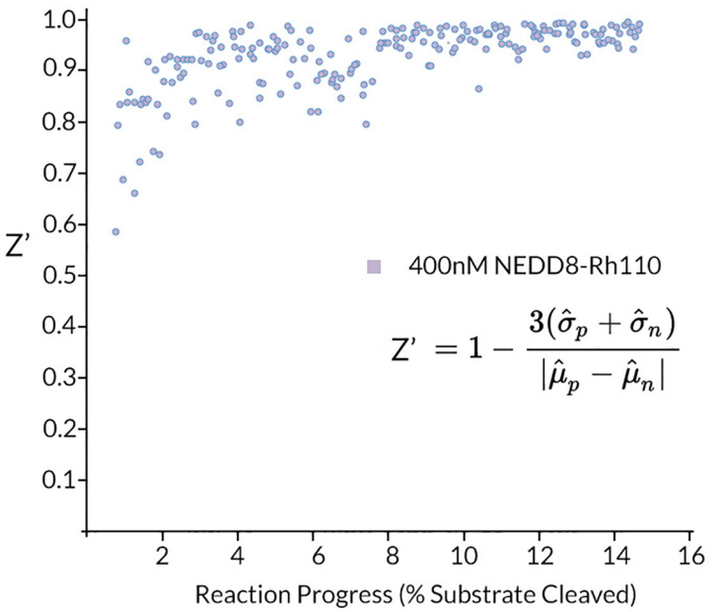
Purity: >97% by LCMS

Excitation/Emission = 485nm/535nm

Storage Buffer: 50mM MES pH 6.0, 150mM NaCl

Store at -80˚C. Avoid multiple freeze thaw cycles.

Quality Control and Performance Data



Robustness of NEDD8-Rhodamine110 in an HTS format.
Fluorescent substrate NEDD8-Rhodamine 110 was incubated with and without 30 pM NEDP1 in a 384 well plate (n = 16), and progress curves were normalized to the maximum fluorescence signal to produce "% reaction progress". The Z' value, a statistical parameter widely used in the evaluation of screening assays, was calculated at each timepoint.

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www.southbaybio.com

Contact:
info@southbaybio.com

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San Jose, CA 95138 USA

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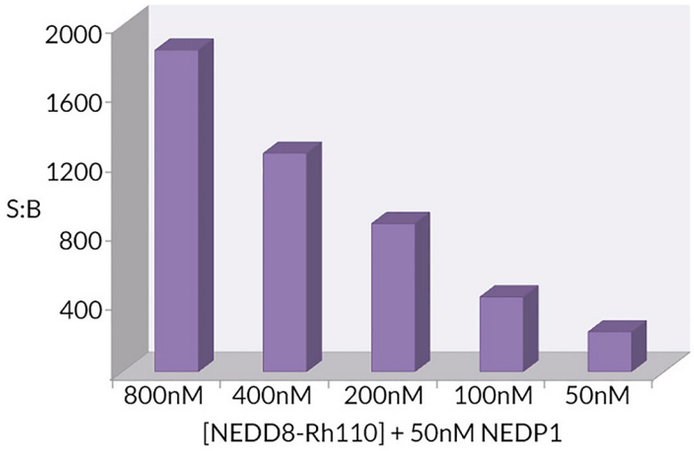
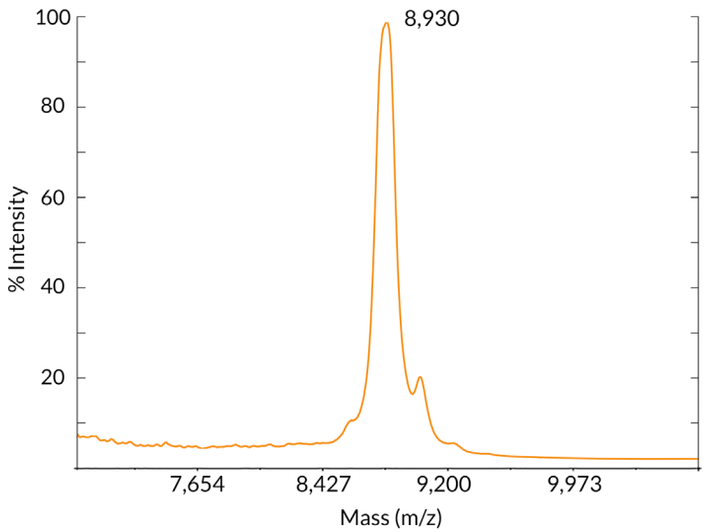


Signal to Background.
The signal to background ratio was determined by 100% hydrolysis of 800nM, 400nM, 200nM, 100nM, and 50nM NEDD8-Rhodamine 110 to liberate the quenched conjugate. Assay Buffer: 50mM HEPES pH7.5, 1mM TCEP, 0.1mg/ml BSA.

Mass Spectrometry Data



References

1) Walden H, Podgorski MS, Huang DT, Miller DW, Howard RJ, Minor DL, Holton JM, Schulman BA (2003). "The structure of the APPBP1-UBA3-NEDD8-ATP complex reveals the basis for selective ubiquitin-like protein activation by an E1". Mol. Cell. 12 (6): 1427–37. PMID 14690597

2) Brown JS, Lukashchuk N, Sczaniecka-Clift M, Britton S, le Sage C, Calsou P, Beli P, Galanty Y, Jackson SP (2015). "Neddylation promotes ubiquitylation and release of Ku from DNA-damage sites". Cell Rep. 11 (5): 704–14. doi:10.1016/j.celrep.2015.03.058. PMID 25921528.

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