NEDD8-Rhodamine 110

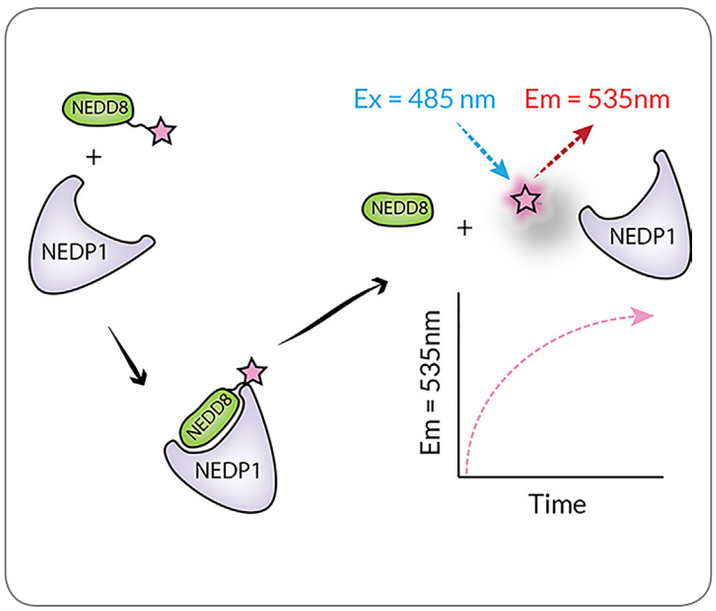
Cat. No. SSB-PS0003
Lot. No. 163060003

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) 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).



Product Information

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

Concentration: 170 µM, 1.5 mg/mL

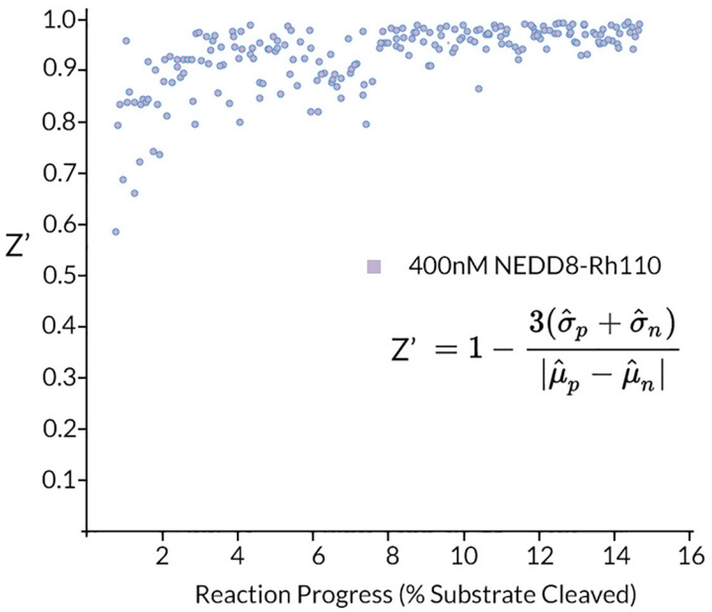
Purity: >97% by LCMS

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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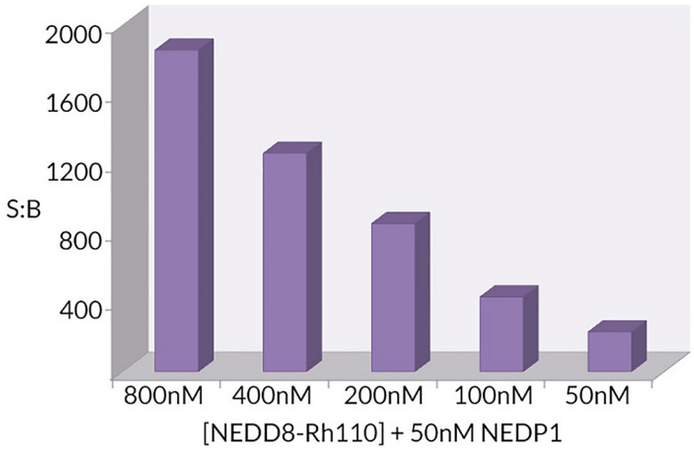
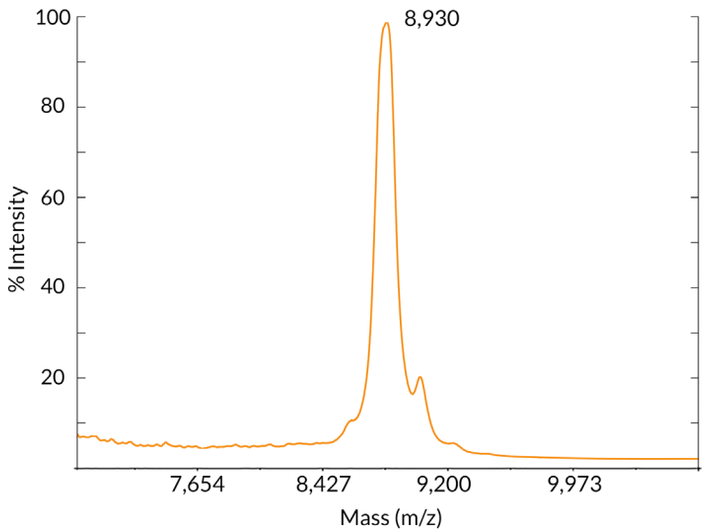


Signal to Background.
The signal to background ratio was determined by 100% hydrolysis of 800nM, 400nM, 200nM, 100nM, and 50nM NEDD8-Rhodamine 110 to liberate the quenched conjugate. Assay Buffer: 50mM HEPES pH7.5, 1mM TCEP, 0.1mg/ml BSA.

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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