

DUB-Glo™ Protease Assay (DUB/SEN/NEDP)

INSTRUCTIONS FOR USE OF PRODUCTS G6260 AND G6261.

Quick
PROTOCOL

Getting Started

This protocol is for performing the DUB-Glo™ Protease Assay in a total volume of 100µl in 96-well plates. The assay can be easily adapted to different volumes provided that the 1:1 ratio of DUB-Glo™ Reagent volume to sample volume is maintained. (e.g., 25µl of sample + 25µl of DUB-Glo™ Reagent in a 384-well plate format).

For the complete DUB-Glo™ Protease Assay protocol, see the *DUB-Glo™ Protease Assay (DUB/SEN/NEDP) Technical Manual*, TM319, available at: www.promega.com/tbs/tm319/tm319.html

Prepare the following reactions to detect DUB/SEN/NEDP enzyme activity (or inhibition of activity) in purified enzyme preparations:

Reaction	Components
Blank	DUB-Glo™ Reagent + protease buffer + vehicle control for test compound or inhibitor (if used)
Positive Control	DUB-Glo™ Reagent + UCH-L3 protease (25–50nM)
Inhibitor Positive Control	DUB-Glo™ Reagent + vehicle control + purified test DUB/SEN/NEDP enzyme
Test Sample	DUB-Glo™ Reagent + test compound + purified test DUB/SEN/NEDP enzyme

Blank is used as a measure of background luminescence associated with the test compound vehicle, and DUB-Glo™ Reagent should be subtracted from experimental values. The **positive control** is used to determine the maximum luminescence obtainable with the purified enzyme system. **Vehicle** refers to the solvent used to dissolve the inhibitor or test compound.

Standard Assay (96-well, 100µl final reaction volume)

1. Add 50µl of DUB-Glo™ Reagent to each well of a white 96-well plate containing 50µl of blank, control or test sample.

Note: If reusing pipette tips, be careful not to touch tips to the well containing sample to avoid cross-contamination.

2. Gently mix contents of wells using a plate shaker at 300–500rpm for 30 seconds. Incubate at room temperature for 10 minutes to 3 hours depending upon convenience of reading time.

Note: Maximal signal is reached typically within ~30 minutes using purified DUB/SEN/NEDP enzyme. Sensitivity is optimal at this time. Temperature fluctuations will affect the luminescent readings; if the room temperature fluctuates too much, a constant-temperature incubator may be desired.

3. Record luminescence with a plate-reading luminometer.

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DUB-Glo™ Protease Assay (continued)

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Assay Tips

1. Prepare the DUB-Glo™ Reagent as described (Section 3, TM319) and mix thoroughly before starting the assay. Allow the reagent to sit at room temperature for at least 30 minutes before use to remove any contaminating free aminoluciferin.
2. The final concentration of DUB/SENP/NEDP should be within the linear range of the assay (see Figure 3, TM319).
3. The recommended DUB/SENP/NEDP dilution buffer is 50mM HEPES (pH 7.2), 10mM DTT, 0.5mM EDTA and 0.1% Prionex® carrier (optional as a carrier if low enzyme concentrations are used).
4. Use identical enzyme concentrations for the assay and positive control reactions.
5. For gentle mixing use a plate shaker.
6. The maximal luminescent signal will be reached in approximately 30 minutes and will have a half-life of several hours (see Figure 4, TM319).

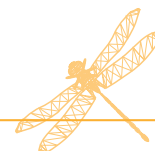
For the complete protocol and ordering information, visit: www.promega.com/tbs/tm319/tm319.html



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