Proteasome Activity Fluorometric Assay Kit

For detecting proteasome activity in cultured cells using our proprietary green fluorescence probe

For research use only - not intended for diagnostic use.

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light.

Materials Supplied

Item	Quantity	Storage Condition
Component A: Proteasome LLVY-R110 Substrate	1 vial	-20°C
Component B: Assay Buffer	10 mL	-20°C
Component C: DMSO	100 µL	-20°C

Δ Note: Thaw all the kit components to room temperature before starting the experiment.

Assay Protocol

Preparation of Cells

For adherent cells: Plate cells overnight in growth medium at 80,000 cells/well/90µL for a 96-well plate or 20,000cells/well/20µL for a 384-well plate.

For non-adherent cells: Centrifuge the cells from the culture medium and then suspend the cell pellet in culture medium at 300,000 cells/well/90µL for a 96-well poly-D lysine plate or 80,000 cells/well/20µL for a 384-well poly-D lysine plate. Centrifuge the plate at 800 rpm for 2 minutes with brake off prior to the experiments.

Δ Note: *Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density*

Preparation of Proteosome Assay Loading Solution

1. Thaw all the kit components at room temperature before use.
2. Make 400X Proteasome LLVY-R110 Substrate stock solution: Add 25 µL of DMSO (Component C) to the vial of Proteasome LLVY-R110 Substrate (Component A), and mix well.
3. Make proteasome assay loading solution: Add 25 µL of 400X Proteasome LLVY-R110 Substrate stock solution (from Step 2) into 10 mL of Assay Buffer (Component B), and mix well.

Δ Note: *25 µL of 400X Proteasome LLVY-R110 Substrate stock solution (from Step 2) and 10 mL of Assay Buffer (Component B) are enough for 1 plate. Aliquot and store the unused 400X Proteasome LLVY-R110 Substrate stock solution and Assay Buffer at -20 °C. Avoid repeated freeze-thaw cycles.*

Run Proteosome Assay:

1. Treat cells with 10 µL of 10X test compound (for a 96-well plate) or 5 µL of 5X test compound (for a 384-well plate) in PBS or desired buffer. For blank wells (medium without the cells), add the corresponding amount of compound buffer.
2. Incubate the cell plates in a 5% CO2, 37 °C incubator for a desired period of time.

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For more details, please visit http://www.apexbt.com/or contact our technical team.

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