ab112154– Proteasome 20S Activity Assay Kit (Fluorometric)

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

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab112154

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.

Δ Note: Pure proteasome or cell lysates can be used directly for screening the proteasome inhibitors.

3. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of proteasome assay loading solution
4. Incubate the plate at 37 °C or room temperature for at least 1 hour (2 hours to overnight), protected from light.
   Δ Note: Each cell line should be evaluated on an individual basis to determine the optimal incubation time.
5. Monitor the fluorescence intensity (top read) at Ex/Em = 490/525 nm.

Data Analysis

The fluorescence in blank wells with the growth medium is subtracted from the values for those wells with the cells. The background fluorescence of the blank wells may vary depending on the sources of the growth media or the microtiter plates

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)