Amplite™ Fluorimetric Proteasome 20S Activity Assay Kit *Green Fluorescence*

Catalog number: 13456
Unit size: 100 tests

Component	Storage	Amount
Component A: Proteasome LLVY-R110 Substrate	Freeze (<-15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Freeze (<-15 °C)	10 mL
Component C: DMSO	Freeze (<-15 °C)	1 vial (100 µL)

OVERVIEW

Our Amplite™ Fluorometric 20S Proteasome Assay Kit is a homogeneous fluorescent assay that measures the chymotrypsin-like protease activity associated with the proteasome complex either in cultured cells or cell lysates. This kit uses LLVY-R110 as a fluorogenic indicator for proteasome activity. Cleavage of LLVY-R110 by proteasome generates the strongly green fluorescent R110 that is monitored fluorimetrically at 520-530 nm with excitation of 480-500 nm. The kit provides all the essential components with an optimized assay protocol. The assay is robust, and can be readily adapted for high-throughput assays for evaluation of proteasome activity or inhibitor screening in cultured cells or in solution. The assay can be performed in a convenient 96-well and 384-well fluorescence microtiter-plate format. The main function of the proteasome is to degrade unneeded or damaged proteins by proteolysis, a chemical reaction that breaks peptide bonds. The proteasomal degradation pathway is essential for many cellular processes, including the cell cycle, the regulation of gene expression, and responses to oxidative stress. The most common form of the proteasome in this pathway is the 26S proteasome, an ATP-dependent proteolytic complex, which contains one 20S (700-kDa) core particle structure and two 19S (700-kDa) regulatory caps. The 20S core contains three major proteolytic activities including chymotrypsin-like, trypsin-like and caspase-like. It is responsible for the breakdown of key proteins involved with apoptosis, DNA repair, endocytosis, and cell cycle control.

AT A GLANCE

Protocol summary

1. Prepare cells with test compounds (100 µL/well/96-well plate or 25 µL/well/384-well plate)
2. Add equal volume of Proteasome working solution (100 µL/well/96-well plate or 25 µL/well/384-well plate)
3. Incubate at 37°C or room temperature for at least 1 hour
4. Monitor fluorescence intensity at Ex/Em = 490/525 nm (Cutoff = 515 nm)

Important Thaw all the kit components at room temperature before use.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	490 nm
Emission:	525 nm
Cutoff:	515 nm
Recommended plate:	Solid black
Instrument specification(s):	Top read mode

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. Proteasome LLVY-R110 Substrate stock solution (400X):
Add 25 µL of DMSO (Component C) to the vial of Proteasome LLVY-R110 Substrate (Component A), and mix well to make 400X Proteasome LLVY-R110 Substrate stock solution.

PREPARATION OF WORKING SOLUTION

Add 25 µL of 400X Proteasome LLVY-R110 Substrate stock solution into 10 mL of Assay Buffer (Component B) and mix well to make Proteasome working solution.

Note This Proteasome working solution is enough for 1 plate. Protect from light.

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit
https://www.aatbio.com/resources/guides/cell-sample-preparation.html

SAMPLE EXPERIMENTAL PROTOCOL

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% CO_2, 37°C incubator for a desired period of time.

   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 working solution.

4. Incubate the plate at 37°C or room temperature for at least 1 hour (2 hours to overnight), protected from light.

   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 (Cutoff = 515 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

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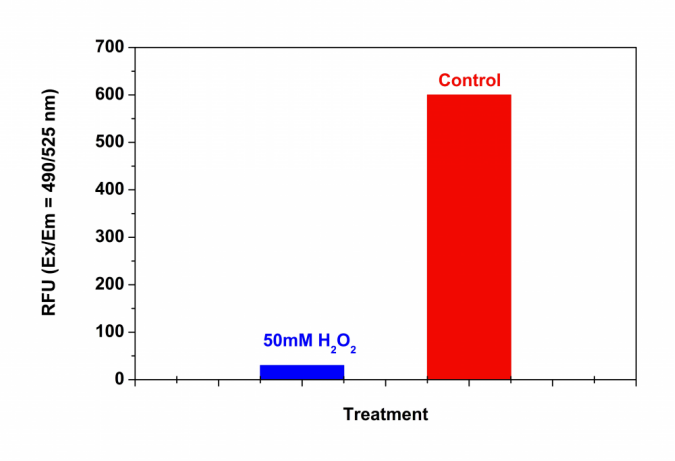

Figure 1. Detection of proteasome activity in Jurkat cells with Amplite™ Fluorimetric Proteasome 20S Activity Assay Kit. Jurkat cells were seeded on the same day at 500,000 cells/90 µL/well in a 96-well black wall/clear bottom Costar plate. The cells were treated with or without 50 mM H_2O_2 for 30 minutes. The proteasome assay loading solution (100 µL/well) was added and incubated in a 5% CO2, 37 °C incubator for 3 hours. The fluorescence intensity was measured at Ex/Em = 490/525 by using a Gemini fluorescent microplate reader (Molecular Devices).

DISCLAIMER