Amplite™ Fluorimetric Proteasome 20S Activity Assay Kit

Green Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 13456 (1 plate)	Keep in freezer Avoid exposure to light	Fluorescence microplate readers

Introduction

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 the responses to oxidative stress. The most common form of the proteasome in this pathway is the proteasome 26S, 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 activities. It is responsible for the breakdown of the key proteins involved with apoptosis, DNA repair, endocytosis, and cell cycle control.

Our Amplite™ Fluorometric Proteasome 20S Assay Kit is a homogeneous fluorescent assay that measures the chymotrypsin-like protease activity associated with the proteasome complex in cultured cells. This kit uses LLVY-R110 as a fluorogenic indicator for proteasome activities. Cleavage of LLVY-R110 by proteasome generates strongly green fluorescent R110 that is monitored fluorimetrically at 520-530 nm with excitation at 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 to evaluate the proteasome activities or screen the inhibitors in cultured cells or in solution. The assay can be performed in a convenient 96-well and 384-well fluorescence microtiter-plate format.

Kit Key Features

Continuous:	Easily adapted to automation with minimal hands on time.
Convenient:	Include all essential assay components.
Increased Sensitivity:	Increase signal to background ratio.
Versatile Applications:	Compatible with many cell lines and targets.

Kit Components

Components	**Amount**
Component A: Proteasome LLVY-R110 Substrate	1 vial
Component B: Assay Buffer	10 mL
Component C: DMSO	1 vial (100 µL)

Assay Protocol (for One plate)

Brief Summary

Prepare cells with test compounds (100 µL/well/96-well plate or 25 µL/well/384-well plate) → Add equal volume of proteasome assay solution (100 µL/well/96-well plate or 25 µL/well/384-well plate) → Incubate at 37 °C or room temperature for at least 1 hour → Monitor fluorescence intensity at Ex/Em = 490/525 nm

Ordering: sales@aatbio.com; Tel: 800-990-8053 or 408-733-1055; Fax: 408-733-1304
Technical Support: support@aatbio.com; Tel: 408-733-1055

1. Prepare cells:

1.1 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.

1.2 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.
Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density.

2. Prepare proteasome assay loading solution:

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

2.2 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.

2.3 Make proteasome assay loading solution: Add 25 μL of 400X Proteasome LLVY-R110 Substrate stock solution (from Step 2.2) into 10 mL of Assay Buffer (Component B), and mix well.
Note: 25 μL of 400X Proteasome LLVY-R110 Substrate stock solution (from Step 2.2) 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 ^{o}C. Avoid repeated freeze-thaw cycles.

3. Run the proteasome assay:

3.1 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.

3.2 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.

3.3 Add 100 μL/well (96-well plate) or 25 μL/well (384-well plate) of proteasome assay loading solution (from Step 2.3).

3.4 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.

3.5 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.

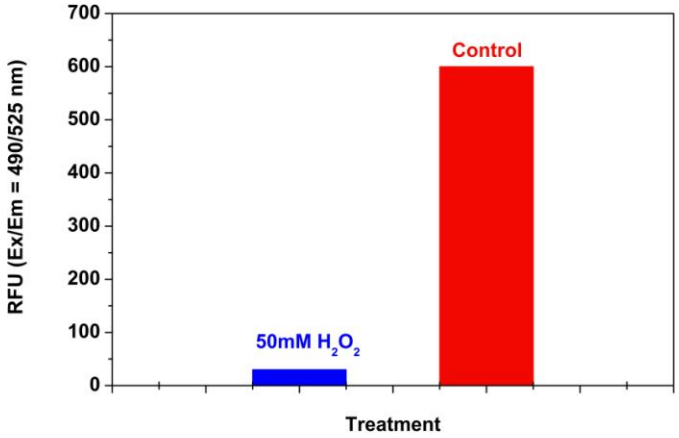

Figure 1. Detection of Proteasome Activity in Jurkat cells. Jurkat cells were seeded on the same day at 500,000 cells/90 µL/well in a 96-well black wall/clear bottom Costar plate. The cells were treated with or without 50 mM H_2O_2 for 30 minutes. The proteasome assay loading solution (100 µL/well) was added and incubated in a 5% CO_2, 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).

References

1. N.A. Osna and T.M. Donohue, World J. Gastroenterol 13 (37), 4931-4937 (2007).
2. P.J. Elliott, T.M. Zollner, and W.-H. Boehnke, J. Mol. Med. 81, 235-245 (2003).
3. D.M. Smith, Z. Wang, A. Kazi et al. J. Mol. Med. 8(7), 382-392 (2002).
4. G. N. DeMartino & C. A. Slaughter; J. Biol. Chem. 274, 22123-22126 (1999).
5. L. Meng, R. Mohan, B. H. B. Kwok et al.; PNAS 96, 10403-10408 (1999)
6. P. Villa, S. H. Kaufmann, W. C. Earnshaw, philos Trans R Soc Lond Biol Sci. 354, 1501-1511 (1999).