ab112154

Proteasome 20S Activity Assay Kit (Fluorometric)

Instructions for Use

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

This product is for research use only and is not intended for diagnostic use.

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1. 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.

ab112154 Proteasome 20S Activity Assay Kit is a homogeneous fluorescent assay that measures the chymotrypsin-like protease activity associated with the proteasome complex in cultured cells. ab112154 uses LLVY-R110 as a fluorogenic indicator for proteasome activities. Cleavage of LLVY-R110 by the proteasome generates strongly green fluorescent R110 that is monitored fluorometrically 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-

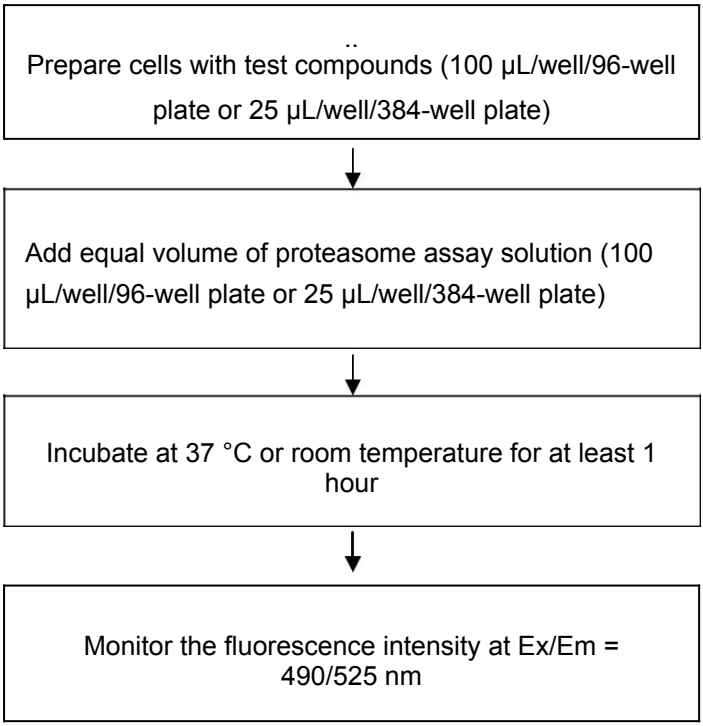
throughput assays to evaluate the proteasome activities or screen inhibitors in cultured cells or in solution. The assay can be performed in a convenient 96-well and 384-well fluorescence microtiter-plate format.

Kit Key Features

- **Continuous:** Easily adapted to automation without a separation step.
- **Convenient:** Includes all essential assay components.
- **Increased Sensitivity:** Increased signal to background ratio.
- **Versatile Applications:** Compatible with many cell lines and targets.

2. Protocol Summary

Summary for One 96-well Plate



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

3. Kit Contents

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

4. Storage and Handling

Keep at -20 °C. Avoid exposure to light.

5. Assay Protocol

Note: This protocol is for one 96 - well plate.

A. Preparation of Cells

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.

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.

B. Preparation of Proteasome 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.

   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.

C. Run Proteasome 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% 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 assay loading solution (from Step B.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.

5. Monitor the fluorescence intensity (top read) at Ex/Em = 490/525 nm.

6. 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

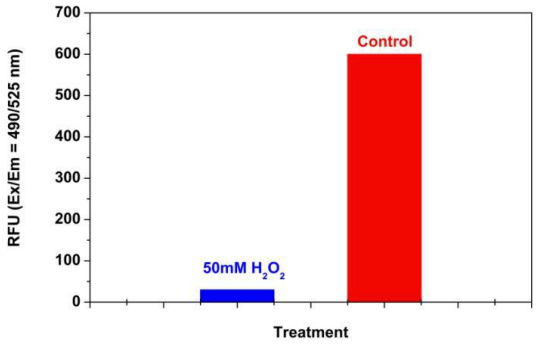


Figure 1. Detection of Proteasome Activity in Jurkat cells. Jurkat cells were seeded on the same day at 500,000 cells/90 µL/well in a 96-well black wall/clear bottom plate. The cells were treated with or without 50 mM H_2O_2 for 30 minutes. The proteasome assay loading solution (100 µL/well) was added and incubated in a 5% CO_2, 37 °C incubator for 3 hours. The fluorescence intensity was measured at Ex/Em = 490/525 using a fluorescent microplate reader.

7. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)*** or ***Deproteinizing sample preparation kit (ab93299)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "contact us" on www.abcam.com for the phone number for your region).

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