20S Proteasome Assay Kit

Item No. 10008041

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -80°C kit. For best results, remove components and store as stated below.

Item Number	Item	100 Tests Quantity	Storage
10011094	20S Proteasome Positive Control	1 vial	-80°C
10011095	20S Proteasome Substrate (SUC-LLVY-AMC)	1 vial	-20°C
10011096	Cell-Based Assay Epigallocatechin Gallate (EGCG)	1 vial	-20°C
10011097	20S Proteasome Assay Buffer	1 vial	RT
10011098	20S Proteasome Lysis Buffer	1 vial	RT
10011297	96-Well Solid Plate (black) with lid	1 plate	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored at -80°C and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. Adjustable pipettes and a repeating pipettor
2. 96-well plate for culturing cells
3. 96-well fluorescent plate reader capable of excitation at 360 nm and emission at 480 nm, respectively
4. A plate centrifuge

INTRODUCTION

Background

The proteasome is a multicatalytic proteinase complex that is involved in the selective degradation of intracellular proteins. The 20S proteasome is the proteolytic core particle of a large protein degradation complex, the 26S proteasome. This 700 kDa protein is ubiquitously distributed in both the cytoplasm and the nucleus in all eukaryotes.[1] The protein is responsible for degrading many cell-cycle control factors, signal transduction factors, transcription factors, and oncogene and anti-oncogene products, thus controlling cell proliferation, differentiation, and apoptosis. Changes in expression of the 20S proteasome have been documented in a number of physiological and pathological conditions such as skeletal muscle atrophy and Alzheimer neurodegeneration.[2] Ethanol inhibits proteasome activity, resulting in the accumulation of insoluble protein aggregates known as Mallory bodies, a common signature of alcoholic liver diseases.[3] On the other hand, proteosome inhibitors exhibit anti-inflammatory and antiproliferative effects, evidence that the proteosome may be an important drug target for the treatment for cancer and inflammatory diseases.[4]

About This Assay

Cayman's 20S Proteasome Assay Kit employs a specific 20S substrate, SUC-LLVY-AMC which, upon cleavage by the active enzyme, generates a highly fluorescent product that can be measured using excitation and emission wavelengths of 360 nm and 480 nm, respectively. The kit is easy to use and can be easily adapted to high-throughput screening for therapeutic compounds regulating activation of the 20S proteasome. A jurkat cell lysate supernatant which contains a high level of 20S activity is included in the kit for use as a positive control. A specific 20S inhibitor, epigallocatechin gallate (EGCG),[5] is also included in the kit to demonstrate specificity of the substrate.

PRE-ASSAY PREPARATION

Reagent Preparation

1. **Substrate (SUC-LLVY-AMC)**

   Add 25 µl of the 20S Substrate (Item No. 10011095) to 1 ml of Assay Buffer (Item No. 10011097). If you are not using all of the 20S Substrate at one time, we recommend that you store the remaining substrate at -20°C.

2. **Positive Control**

   This vial contains 500 µl of Jurkat cell lysate supernatant which contains a high level of 20S activity.

   To use the lysate supernatant as a positive control, add 90 µl of this control directly into corresponding wells in the assay plate.

   To run a positive control curve using this lysate, obtain six clean test tubes and label them #1 to # 6. Add 250 µl of 20S Proteasome Lysis Buffer (Item No. 10011098) into tubes #1-#6. Transfer 250 µl of Positive Control (Item No. 10011094) into tube #1 and mix thoroughly. Serially dilute the control by removing 250 µl from tube #1 and placing it into tube #2; mix thoroughly. Next remove 250 µl from tube #2 and place it into tube #3; mix thoroughly. Repeat this procedure for tubes #4 and #5. Do not add any control to tube #6. This tube will be your blank.

3. **20S Inhibitor Solution**

   If you are not using all of the 20S inhibitor at one time, we recommend that you make small aliquots and store them at -20°C. Dilute the 20S Inhibitor (Item No. 10011096) 1:10 in the Assay Buffer prior to use in the assay.

ASSAY PROTOCOL

Plate Configuration

There is no specific pattern for using the wells on the plate. A typical experimental plate will include wells without cells, wells with cells treated with experimental compounds and wells of untreated cells. We recommend that each treatment be performed in triplicate and that you record the contents of each well on the template sheet provided (see page 14).

Pipetting Hints

- Use a repeating pipettor to deliver reagents to the wells. This saves time and helps to maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

Performing the Assay

1. Seed cells in a 96-well plate at a density of 10^5-10^6 cells/well in 100 µl of culture medium. Incubate overnight in a CO_2 incubator at 37°C. The next day, treat the cells with compounds to be tested, or vehicle, and continue to culture the cells at 37°C for a period of time according to your protocol. We recommend that you do triplicate wells for each treatment.
2. Centrifuge the plate in a plate centrifuge at 500 x g for five minutes.
3. Aspirate the culture medium.
4. Add 200 µl of the 20S Proteasome Assay Buffer (Item No. 10011097) to each well and centrifuge the plate in a plate centrifuge at 500 x g for five minutes.
5. Aspirate the supernatant.
6. Add 100 µl of the 20S Proteasome Lysis Buffer (Item No. 10011098) to each well.
7. Incubate with gentle shaking on an orbital shaker for 30 minutes at room temperature.
8. Centrifuge the plate in a plate centrifuge at 1,000 x g for 10 minutes. Transfer 90 µl of the supernatant from each well to a corresponding well in the black 96-well plate included in the kit. Add 10 µl of Assay Buffer (sample activity measurement) or 10 µl of the 20S Inhibitor Solution (to test assay specificity) to appropriate wells.
9. Add 100 µl of Positive Control to corresponding wells of the black plate.
10. Add 10 µl of the Substrate Solution to each well and incubate the plate at 37°C for one hour.
11. Read the fluorescent intensity of each well (excitation = 360 nm; emission = 480 nm).

PERFORMANCE CHARACTERISTICS

Sample Data

Examples of typical data obtained with this assay are shown in the figures below. Your data will vary depending on the cell line and culture conditions used.

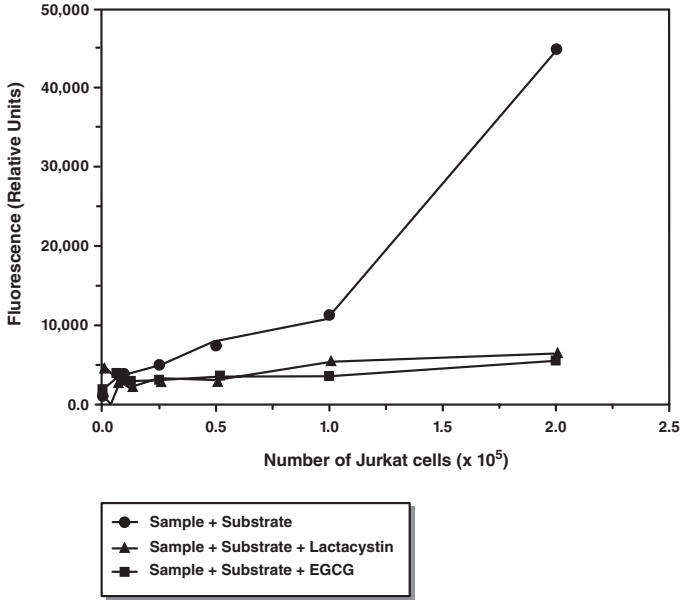


Figure 1: 20S Proteasome Activity in Jurkat Cell Lysate Supernatant. Jurkat cells were seeded in a 96-well plate in 100 µl of culture medium at a density indicated on the graph. Cells were then processed for measurement of 20S activity according to the protocol described in the **Performing the Assay** section. Relative fluorescent intensity was then measured with a plate reader. *Note: Lactacystin is a known selective 20S inhibitor.*

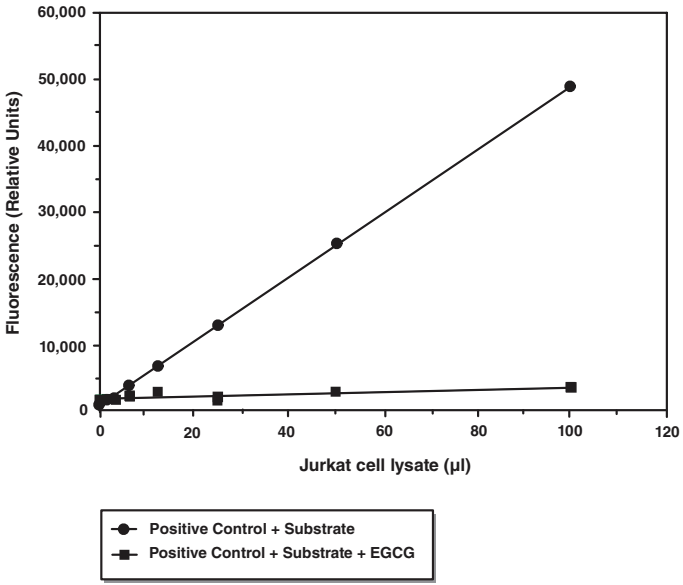


Figure 2: Positive Control Curve. The Jurkat cell lysate was serially diluted as described in the **Preparation of Reagents** section and the 20S proteasome activity was measured according to the protocol described in the **Performing the Assay** section. Relative fluorescent intensity was then measured with a plate reader.

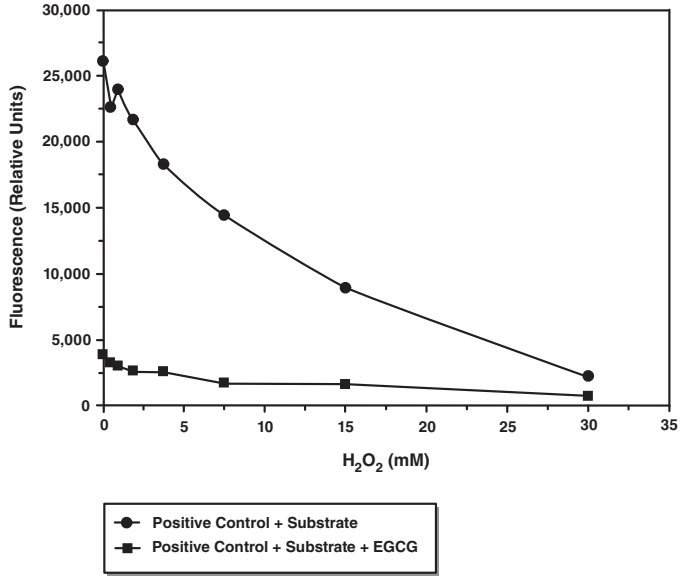


Figure 3: H_2O_2 inhibits 20S Proteasome Activity in Raw 264.7 cells. Raw 264.7 cells were seeded in a 96-well plate in 100 µl of culture medium at 8 x 10^4 cells/well and grown in DMEM containing 10% FBS for three days. Cells were then treated with different doses of H_2O_2 as indicated on the chart for 30 minutes and processed for measurement of 20S activity according to the protocol described in the **Performing the Assay** section. Relative fluorescent intensity was then measured with a plate reader.

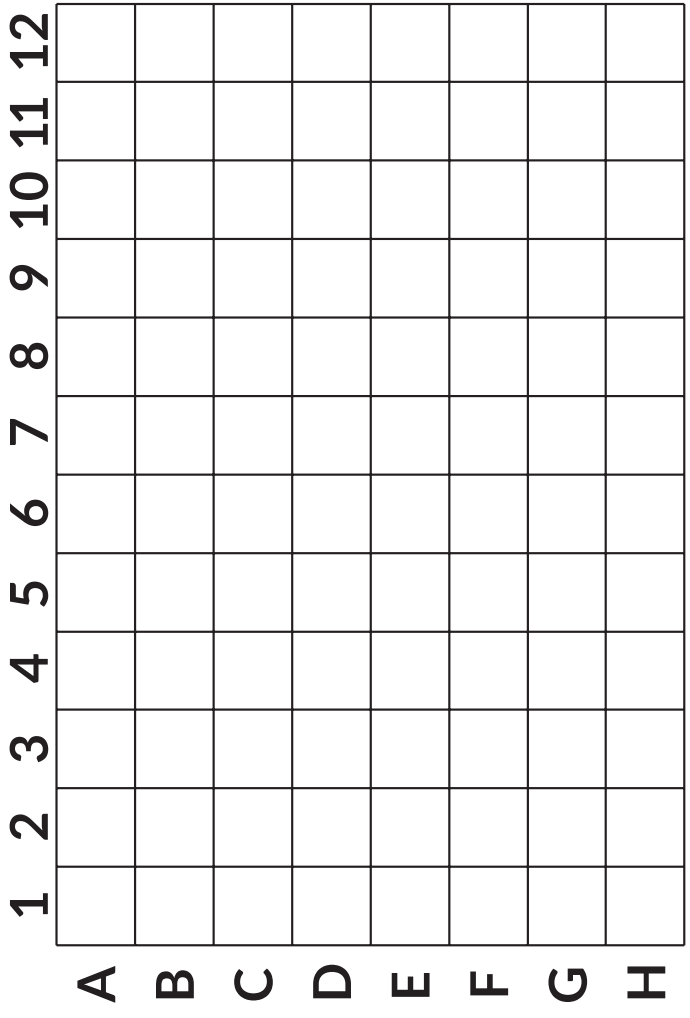
RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates	A. Poor pipetting/technique B. Bubbles in the well	A. Use a repeating pipettor B. Make sure every well contains no bubbles
High reading in all wells	Phenol red residue in the wells	Carefully aspirate all the culture medium and wash the wells with the Assay Buffer throughly
Erratic response curve of compound treatments	A. Cells lost from wells during processing B. Unequal number of cells in each well	A. Do triplicate wells for each treatment. Use only healthy cells at the beginning of the experiment B. Make sure each well contains the same number of cells

References

1. Zwickl, P., Voges, D., and Baumeister, W. The proteasome: a macromolecular assembly designed for controlled proteolysis. *Philos Trans R Soc Lond B Biol Sci.* **354**, 1501-1511 (1999).
2. DeMartino, G.N. and Slaughter, C.A. The proteasome, a novel protease regulated by multiple mechanisms. *J. Biol. Chem.* **274(32)**, 22123-22126 (1999).
3. Osna, N.A. and Donohue, T.M., Jr. Implication of altered proteasome function in alcoholic liver injury. *World J. Gastroenterol* **13(37)**, 4931-4937 (2007).
4. Elliott, P.J., Zollner, T.M., and Boehncke, W.-H. Proteasome inhibition: A new anti-inflammatory strategy. *J. Mol. Med.* **81**, 235-245 (2003).
5. Smith, D.M., Wang, Z., Kazi, A., *et al.* Synthetic analogs of green tea polyphenols as proteasome inhibitors. *Mol. Med.* **8(7)**, 382-392 (2002).



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.