



# 20S Proteasome Assay Kit

Catalog Number KA1352

96 assays

Version: 06

Intended for research use only

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## **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.<sup>1</sup> 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.<sup>2</sup> Ethanol inhibits proteasome activity, resulting in the accumulation of insoluble protein aggregates known as Mallory bodies, a common signature of alcoholic liver diseases.<sup>3</sup> On the other hand, proteasome inhibitors exhibit anti-inflammatory and antiproliferative effects, evidence that the proteasome may be an important drug target for the treatment for cancer and inflammatory diseases.<sup>4</sup>

### **Principle of the Assay**

The 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),<sup>5</sup> is also included in the kit to demonstrate specificity of the substrate.

## General Information

### Materials Supplied

List of component

Item	Amount
20S Proteasome Positive Control	1 vial
20S Proteasome Substrate (SUC-LLVY-AMC)	1 vial
Cell-Based Assay Epigallocatechin Gallate (EGCG)	1 vial
20S Proteasome Assay Buffer	1 vial
20S Proteasome Lysis Buffer	1 vial
96-Well Solid Plate (black) with lid	1 plate

### Storage Instruction

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

Item	Storage
20S Proteasome Positive Control	-80°C
20S Proteasome Substrate (SUC-LLVY-AMC)	-20°C
Cell-Based Assay Epigallocatechin Gallate (EGCG)	-20°C
20S Proteasome Assay Buffer	Room Temperature
20S Proteasome Lysis Buffer	Room Temperature
96-Well Solid Plate (black) with lid	Room Temperature

### Materials Required but Not Supplied

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

### Precautions for Use

WARNING: This product is for laboratory research use only: Not for human or veterinary diagnostic or therapeutic use.

- ✓ 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.

✓ 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.

## Assay Protocol

### Reagent Preparation

✓ Substrate (SUC-LLVY-AMC)

Add 25  $\mu\text{L}$  of the 20S Substrate to 1 mL of Assay Buffer. If you are not using all of the 20S Substrate at one time, we recommend that you store the remaining substrate at  $-20^{\circ}\text{C}$ .

✓ Positive Control

This vial contains 500  $\mu\text{L}$  of Jurkat cell lysate supernatant which contains a high level of 20S activity.

To use the lysate supernatant as a positive control, add 90  $\mu\text{L}$  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  $\mu\text{L}$  of 20S Proteasome Lysis Buffer into tubes #1-#6. Transfer 250  $\mu\text{L}$  of Positive Control into tube #1 and mix thoroughly. Serially dilute the control by removing 250  $\mu\text{L}$  from tube #1 and placing it into tube #2; mix thoroughly.

Next remove 250  $\mu\text{L}$  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.

✓ 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  $-20^{\circ}\text{C}$ . Dilute the 20S Inhibitor 1:10 in the Assay Buffer prior to use in the assay.

### Assay Procedure

1. Seed cells in a 96-well plate at a density of  $10^5$ - $10^6$  cells/well in 100  $\mu\text{L}$  of culture medium. Incubate overnight in a  $\text{CO}_2$  incubator at  $37^{\circ}\text{C}$ . The next day, treat the cells with compounds to be tested, or vehicle, and continue to culture the cells at  $37^{\circ}\text{C}$  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  $\mu\text{L}$  of the 20S Proteasome Assay Buffer 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  $\mu\text{L}$  of the 20S Proteasome Lysis Buffer 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  $\mu\text{L}$  of the supernatant from each well to a corresponding well in the black 96-well plate included in the kit. Add 10  $\mu\text{L}$  of Assay Buffer (sample activity measurement) or 10  $\mu\text{L}$  of the 20S Inhibitor Solution (to test assay specificity) to

appropriate wells.

9. Add 100  $\mu$ L of Positive Control to corresponding wells of the black plate.
10. Add 10  $\mu$ L 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).

## Data Analysis

### Calculation of Results

#### ✓ 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.

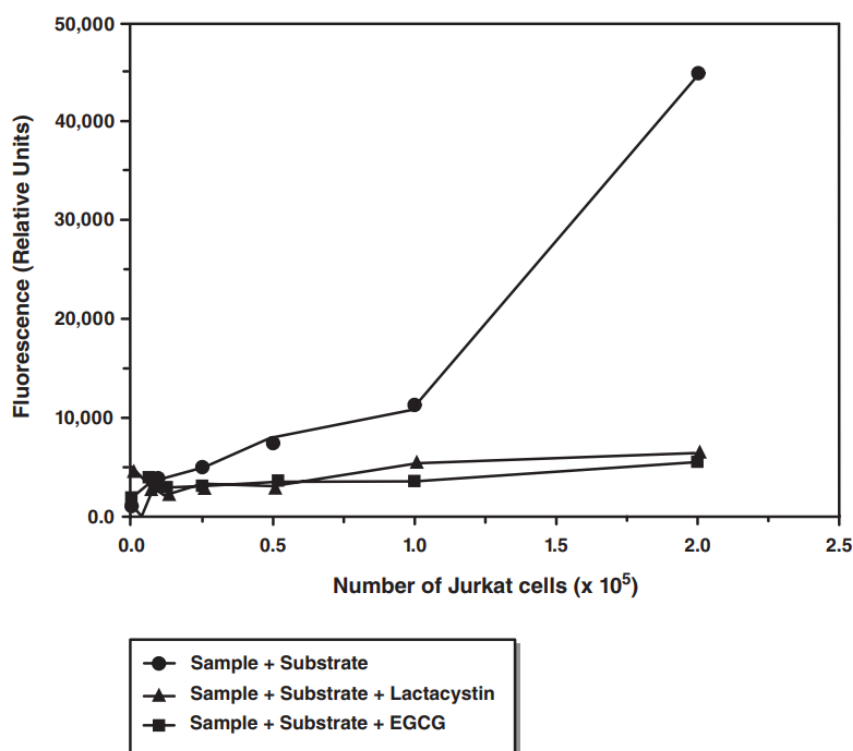


Figure 1: 20S Proteasome Activity in Jurkat Cell Lysate Supernatant.

Jurkat cells were seeded in a 96-well plate in 100  $\mu$ L 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 Assay Procedure section. Relative fluorescent intensity was then measured with a plate reader.

*Note: Lactacystin is a known selective 20S inhibitor.*

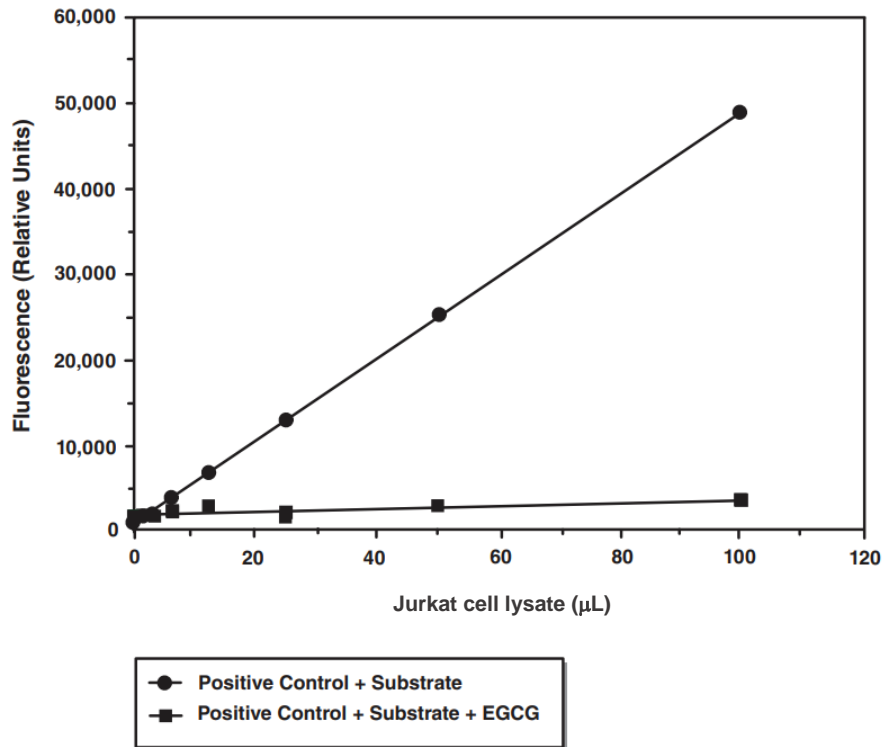


Figure 2: Positive Control Curve.

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.

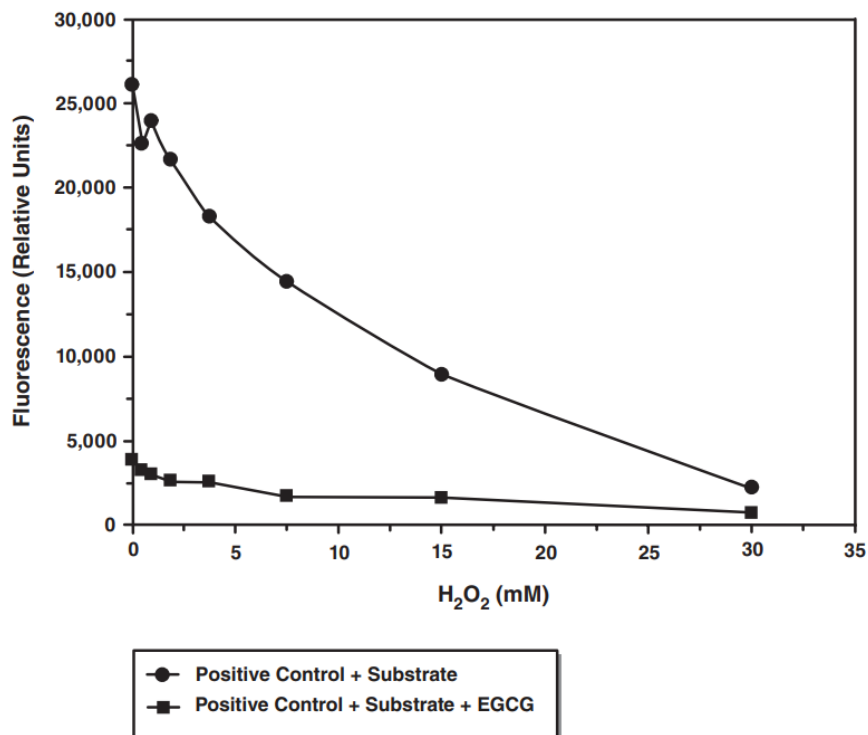


Figure 3: H<sub>2</sub>O<sub>2</sub> inhibits 20S Proteasome Activity in Raw 264.7 cells.

Raw 264.7 cells were seeded in a 96-well plate in 100  $\mu$ L of culture medium at  $8 \times 10^4$  cells/well and grown in DMEM containing 10% FBS for three days. Cells were then treated with different doses of H<sub>2</sub>O<sub>2</sub> 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

<b>Problem</b>	<b>Possible Causes</b>	<b>Recommended Solutions</b>
Erratic values; dispersion of duplicates	A. Poor pipetting / technique. B. Bubbles in the well.	A. Use a repeat 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 thoroughly.
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

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**Plate Layout**

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	A	B	C	D	E	F	G	H