20S Proteasome Activity Assay Kit

For 100 Assays

Cat. No. APT280

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

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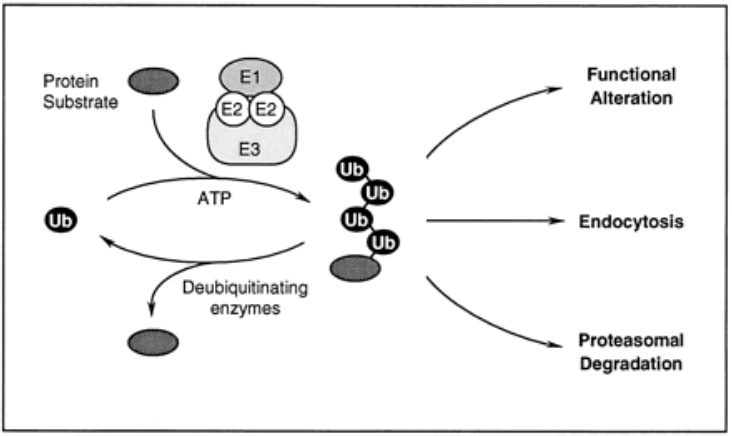
Introduction

The ubiquitin-proteasome pathway is the major proteolytic system in the cytosol of eukaryotic cells, where it catalyzes the selective degradation of short-lived regulatory proteins and the rapid elimination of proteins with abnormal conformation (1-2). The critical protease in this pathway is the 26S proteasome, an ATP-dependent proteolytic complex, which is formed by the association of the barrel-shaped 20S proteasome (700-kDa) and two 19S (700-kDa) regulatory complexes (3-4). The 20S Proteasome, catalytic core of the proteasome complex, is responsible for the breakdown of key proteins involved with apoptosis, DNA repair, endocytosis, and cell cycle control (4-6). The CHEMICON® Proteasome Activity Assay Kit provides a quick, efficient and sensitive system for evaluation of proteasome activity in cell lysates or inhibitor screening.

Figure 1: Protein Ubiquitination and Proteasomal Degradation



Test Principle

CHEMICON®'s Proteasome Activity Assay Kit provides a simple and convenient means for assaying proteasome activity that recognize the substrate LLVY (7). The assay is based on detection of the fluorophore 7-Amino-4-methylcoumarin (AMC) after cleavage from the labeled substrate LLVY-AMC. The free AMC fluorescence can be quantified using a 380/460 nm filter set in a fluorometer.

A proteasome inhibitor, Lactacystin, is included as a test inhibitor for screening purpose. Lactacystin is a microbial natural product and the most specific and potent inhibitor of proteasomes currently known (8). Assay buffer contains Sodium Dodecyl Sulfate (SDS) for activation of the 20S Proteasome.

Application

The Proteasome Activity Assay Kit is a relatively quick method for detection of intracellular proteasome activity. Testing of purified proteasome enzyme, *in vitro* inhibitor screening and the study of proteasome regulation can be performed with this assay.

The CHEMICON® Proteasome Activity Assay Kit is intended for research use only, not for diagnostic or therapeutic applications.

Kit Components

1. 20S Proteasome Positive Control (Part No. 90205): One vial containing 25 µL 20S Proteasome Positive Control in 50mM HEPES, pH 7.6, 150 mM NaCl and 1mM DTT.
2. 10X Assay Buffer (Part No. 90209): One 1.5 mL vial containing 250mM HEPES, pH 7.5, 5mM EDTA, 0.5% NP-40, and 0.01% SDS (w/v).
3. Proteasome Substrate (Suc-LLVY-AMC) (Part No. 90206): One vial containing 500 µg peptide substrate.
4. Lactacystin (20S Proteasome Inhibitor) (Part No. 90208): One vial containing 9.4 µg inhibitor.
5. AMC Standard (Part No. 90207): One vial containing 2.2 µg AMC standard.

Materials Not Supplied

- 96-well fluorometer plate
- Adjustable volume pipettor with disposable tips
- 37°C incubator
- DMSO
- Fluorometer with a 380/460nm filter set

Storage

The 20S Proteasome Activity Assay Kit is a dual temperature storage kit. Store kit components at the temperatures indicated on the labels and insert until their expiration date.

- Store 20S Proteasome Positive Control at -80°C up to its expiration date. Avoid repeated freeze-thaw cycles.
- Store all other components at -20°C up to their expiration dates. Avoid repeated freeze-thaw cycles.

Preparation of Reagents

1. 20S Proteasome Positive Control: Thaw vial at 2-8°C. Aliquot and store at -70°C up to the vial's expiration date. Avoid multiple freeze thaws.
2. 10X Assay Buffer: Dilute the 10X Assay Buffer with deionized water. Stir to homogeneity and store at 4°C up to its expiration date.
3. Proteasome Substrate: Reconstitute with 65 µL DMSO (giving a 10 mM stock solution). Aliquot and store at -20°C up to 3 months. Avoid multiple freeze thaws.
4. AMC Standard: Reconstitute with 100 µL DMSO (giving a 125 µM stock solution). Aliquot and store at -20°C up to 6 months. Avoid multiple freeze thaws.

   Note: *Both Substrate and AMC Standard are light sensitive; amber vials or equivalent should be used.*
5. Lactacystin: Reconstitute with 10 µL DMSO (giving a 2.5 mM stock solution). Aliquot and store at -20°C up to 1 month. Avoid multiple freeze thaws.

Generating an AMC Standard Curve

Prepare a dilution series (1:2 is suggested) of AMC Standard in the concentration range of 0.04 µM – 12.5 µM by diluting the reconstituted AMC Standard (see above) in 1X Assay Buffer. Add 100 µL of each dilution to a well. Include 100 µL of 1X Assay Buffer as a blank. Read fluorescence using a 380/460 nm filter set in a fluorometer.

20S Proteasome Positive Control

1. Thaw 20S Proteasome Positive Control at 2-8°C.

   Note: *For best stability, only dilute the required volume of 20S Proteasome Positive Control; retain the rest as stock solution.*

2. Perform a dilution series of Proteasome Positive Control (1:4 to 1:256 is suggested) by diluting the stock solution in 1X Assay Buffer.

   Note: *Only 10 µL of each dilution is required per well.*

3. Transfer 10 µL of each dilution to a 96-well fluorometer plate. To each well, add 80 µL of 1X Assay Buffer.

4. Finally, dilute the Proteasome Substrate stock solution (see Preparation of Reagents) 1:20 in 1X Assay Buffer and add 10 µL to each well. Mix well and incubate 1-2 hrs at 37°C to 60°C.

   Note: *For best stability, only dilute the required volume of Proteasome Substrate; retain the rest as stock solution.*

5. Record fluorescence using a 380/460 nm filter set in a fluorometer.

Assay Instructions

- Proteasome Substrate stock solution (see Preparation of Reagents) must be diluted 1:20 in 1X Assay Buffer prior to usage.

  Note: *For best stability, only dilute the required volume of Proteasome Substrate; retain the rest as stock solution.*

- Lactacystin stock solution (see Preparation of Reagents) must be diluted 1:10 in 1X Assay Buffer prior to usage.

  Note: *For best stability, only dilute the required volume of Lactacystin; retain the rest as stock solution.*

Sample	Assay Mixture				Proteasome Substrate	Total Volume
	10X Assay Buffer	**Proteasome Sample**	**Inhibitor**	**DI H_2O**		
Buffer Blank	10 µL	0 µL	0 µL	90 µL	0 µL	100 µL
Substrate Blank	10 µL	0 µL	0 µL	80 µL	10 µL	100 µL
Test Sample	10 µL	X µL	0 µL	80-X µL	10 µL	100 µL
Test Sample + Inhibitor (optional)	10 µL	X µL	Y µL	80-(X+Y) µL	10 µL	100 µL

1. Prepare assay mixture in a 96-well fluorometer plate or standard microcentrifuge tubes, according to the above table. If using the optional inhibitor, pre-incubate inhibitor with proteasome sample for 15 minutes at room temperature before adding proteasome substrate.
2. Incubate samples for 1-2 hours at 37ºC to 60ºC.
3. Read fluorescence using a 380/460 nm filter set in a fluorometer.

   Note: *Background reading from cell lysates and buffers should be subtracted from the readings before calculating fold increase in proteasome activity.*

Calculation of Results

The following charts illustrate typical results including dilutions of the AMC Standard and the activity curve of 20S Proteasome Positive Control contained in the kit. Fluorescence data was collected using a PE Biosystems CytoFluor 4000 plate reader using a 380 nm excitation and 460 nm emission filters at a voltage gain setting of 50. One should use the data below for reference only. This data should not be used to interpret actual assay results.

Figure 1: AMC Fluorogenic Standard Curve

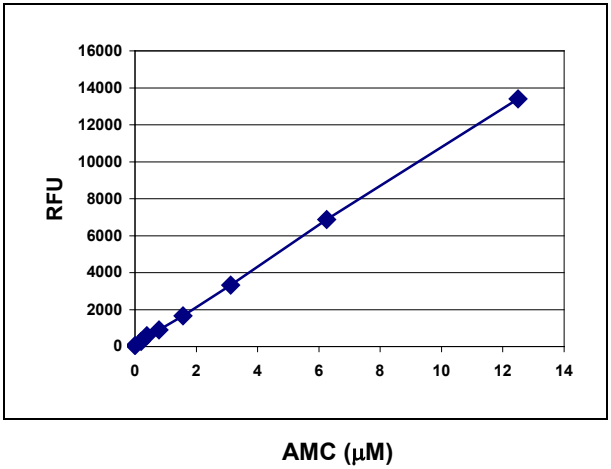


Figure 2: Activity Curve of 20S Proteasome Positive Control. 20S Proteasome Positive Control was incubated for 2 hours at 37°C.

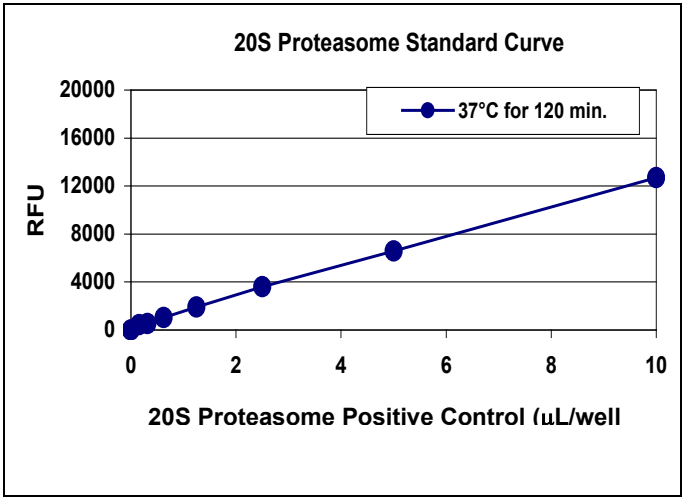
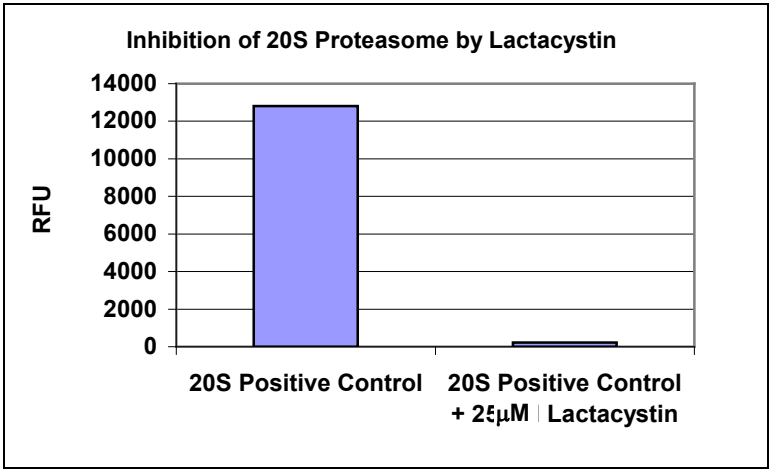


Figure 3: Inhibition of 20S Proteasome by Lactacystin. 10 µg of the 20S Proteasome Positive Control was incubated with Lactacystin for 10 minutes at room temperature. Proteasome Activity Assay is performed according to the Assay Instructions.



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Cat No. APT280

September 2012
Revision E: 41319