Proteasome Activity Assay Kit

Catalog Number KA1431

100 assays

Version: 03

Intended for research use only

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Introduction

Background

Proteasomes are very large (20S, 26S) protein assemblies found in both the nucleus and cytoplasm of all eucaryotes (and in some procaryotes). They are responsible for the degradation and recycling of proteins which have been previously tagged with ubiquitin. Such tagged proteins are degraded into peptides approximately 7-8 amino acids long which are subsequently further degraded. The 20S assembly is the functional protease structure with chymotrypsin-like, trypsin-like and caspase-like protease activities. Proteasome Activity Assay Kit takes advantage of the chymotrypsin-like activity, utilizing an AMC-tagged peptide substrate which releases free, highly fluorescent AMC in the presence of proteolytic activity. The kit also includes a positive control (Jurkat Cell lysate with significant proteasome activity) and a specific proteasome inhibitor MG-132 which suppresses all proteolytic activity due to proteasomes. This permits differentiation of proteasome activity from other protease activity which may be present in samples.

General Information

Materials Supplied

List of component

Component	Amount
Proteasome Assay Buffer	25 ml
Proteasome Substrate (Succ-LLVY-AMC in DMSO)	100 µl
Proteasome Inhibitor (MG-132 in DMSO)	100 µl
AMC Standard (1 mM in DMSO)	100 µl
Positive Control (lyophilized)	1 vial

Storage Instruction

Store the kit at -20℃, protect from light. Read the entire protocol before performing the assay. Avoid repeated freeze/thaw cycles. All samples and the Positive Control should be assayed in duplicate, (once in the absence and once in the presence of the Proteasome Inhibitor). An opaque white micro-well plate is recommended. This protocol is designed for use in a 96 well plate. 384-well plates can also be used but all reagent amounts should be reduced 5-fold (diluted if necessary). Do not use protease inhibitors during cell lysate preparation.

Precautions for Use

- Proteasome Substrate, Proteasome Inhibitor, AMC Standard
  Ready to use as supplied. These DMSO solutions must be warmed to room temperature prior to use to melt frozen DMSO. We recommend briefly warming in a 37℃ water bath, pipetting up and down to ensure they are completely melted and mixed before use. Store at -20℃ in the dark when not in use.

- Positive Control
  Reconstitute with 100 µl dH2O. If kit will be used multiple times over an extended period of time, aliquot portions and store at -80℃. Keep on ice while in use. Avoid multiple freeze/thaw cycles.

Assay Protocol

Assay Procedure

1. AMC Standard Preparation: Dilute AMC standard 100-fold (10 µl + 990 µl dH2O) than add 0, 2, 4, 6, 8, 10 µl of AMC standard to a series of microplate wells. Adjust volume to 100 µl/well with Assay Buffer to generate 0, 20, 40, 60, 80 and 100 pmol per well AMC Standard.
2. Positive Control Preparation: Add 10 µl of the positive control to paired wells. Bring volume to total 100 µl by adding 90 µl of Assay Buffer to each well.
3. Samples: Prepare by homogenizing cells with 0.5% NP-40 in dH_2O or PBS. Add up to 50 µl of each cell extract or other proteasome sample to be tested to paired wells. Bring the volume of each well to 100 µl with Assay Buffer.
4. Inhibitor: Add 1 µl of the Proteasome inhibitor to one of the paired wells, 1 µl of Assay Buffer to the other well, mix.
5. Substrate: Add 1 µl of Proteasome Substrate to all wells, mix, protected from light, mix.
6. Read: Measure kinetics of fluorescence development at Ex/Em 350/440 nm in a micro-plate reader at 37℃ for 30-60 minutes. There is a slight lag and nonlinearity to the kinetics due to the time it takes for the reaction mix to warm up to 37℃. Measurement of the wells which do not contain Proteasome Inhibitor will show total proteolytic activity RFU_1 and the wells containing Proteasome Inhibitor will show non-proteasome activity $iRFU_1$ at T_1, Measure RFU_2 and $iRFU_2$ again at T_2 after 30 min (or longer time if the sample activity is low). The RFU generated by proteasome is $\Delta RFU = (RFU_2 - iRFU_2) - (RFU_1 - iRFU_1)$.

   Note: It is essential to read RFU_1, $iRFU_1$, RFU_2 and $iRFU_2$ in the linear reaction range. It will be more accurate if you monitor the reaction kinetics as shown in Fig. 1B. Then choose T_1 and T_2 in the appropriate linear range. From our experience, RFU1 and iRFU1 should be measured after ~20-25 minutes.

Data Analysis

Calculation of Results

Plot the AMC Standard Curve. Apply the ΔRFU to the AMC Standard Curve to get B nmol of AMC (amount generated between T_1 and T_2 in the reaction wells specifically by proteasome activity).

$$\text{Proteasome Activity} = \frac{B}{(T2 - T1) \times V} \times \text{Sample Dilution Factor} = \text{nmol/min/ml} = \text{U/ml}$$

Where: B is the AMC amount from AMC Standard Curve (in nmol).

T_1 is the time of the first reading (RFU_1 and $iRFU_1$) (in min).

T_2 is the time of the second reading (RFU_2 and $iRFU_2$) (in min).

V is the pretreated sample volume added into the reaction well (in ml).

Proteosome Unit Definition: One unit proteasome activity is defined as the amount of proteasome which generates 1.0 nmol of AMC per minute at 37℃.

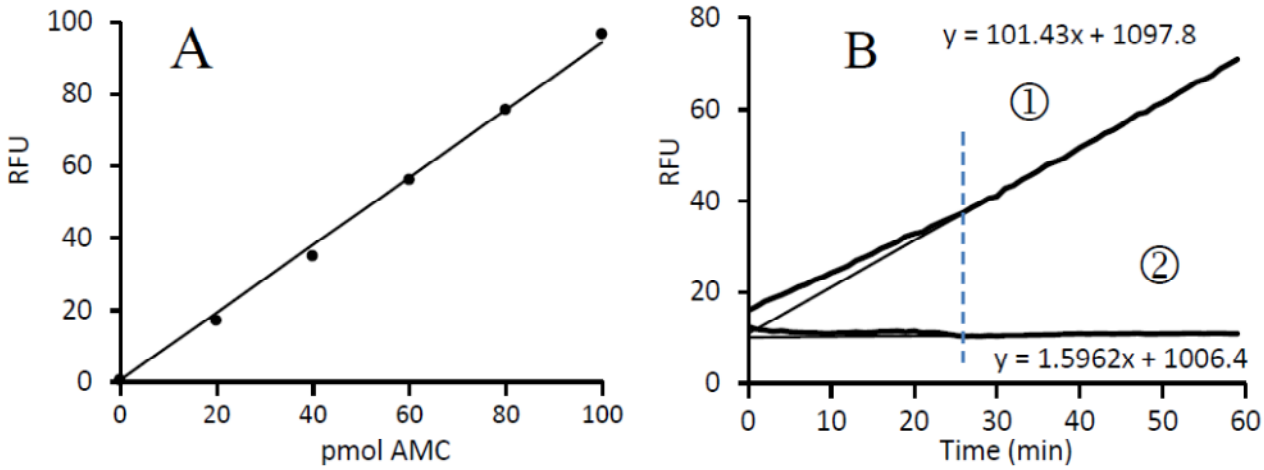

Fig. 1. AMC Standard Curve and Proteasome Activity assay using the kit protocol:
A: AMC standard curve 0 -100 pmole; B: Kinetics of Proteasome Activity assay in the absence 1 and presence 2 of MG-132 Proteasome inhibitor. Equations represent best fit of lines during the linear portion of the reaction (after ~ 25 minutes in this case).