

ab303734 – 20S Immunoproteasome Activity Assay Kit (Fluorometric)

For measuring 20S immunoproteasome in tissue lysate, cell lysate, and purified protein.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

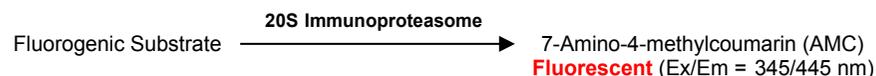
<http://www.abcam.com/ab303734>

Storage and Stability

The entire Assay kit may be stored at -20°C protected from light.

Introduction:

Proteasome mediated protein degradation is one of the major pathways for the degradation of damaged, misfolded or aggregated proteins in cells. The 26S proteasome is the most common form of the proteasome that degrades all ubiquitinated proteins. It is an ATP-dependent proteolytic complex containing a barrel-shaped core (20S proteasome) and two caps (19S regulatory complexes) at both ends. The 20S immunoproteasome is a specialized form of the proteasome, which is expressed during oxidative stress or by stimulation from proinflammatory cytokines. During oxidative stress and inflammatory processes, the catalytic subunits $\beta 1$, $\beta 2$ and $\beta 5$ of the 20S proteasome core are replaced by the inducible subunits $\beta 1i/LMP2$, $\beta 2i/MECL1$ and $\beta 5i/LMP7$, respectively. This allows the 20S immunoproteasome to cleave proteins differently as compared to the 20S proteasome due to a shift towards chymotrypsin-like catalytic activity. Additionally, these inducible catalytic subunits play an important role in the generation of new antigenic peptides for presentation via the major histocompatibility class I pathway. **BioVision's 20S Immunoproteasome Activity Assay Kit** uses a plate-based fluorometric assay to measure the activity of the 20S immunoproteasome. In this assay, the 20S immunoproteasome cleaves a fluorogenic substrate to release 7-amino-4-methylcoumarin, a fluorescent compound that produces a stable signal at Ex/Em = 345/445 nm. The kit can detect as low as 0.02 μ M (0.02 pmol substrate proteolysis per minute) of 20S immunoproteasome under assay conditions.



Materials Supplied

Item	Quantity
20S IP Assay Buffer	25 ml
20S IP Substrate (Avoid light)	200 μ l
20S IP Positive Control	20 μ l
AMC Standard	100 μ l
20S IP Inhibitor	100 μ l

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 96-well white plate with flat bottom
- Multi-well spectrophotometer

Reagent Preparation

- Upon arrival, store the kit at -20 °C, protected from light.
- Briefly centrifuge all small vials before opening.
- Read the entire protocol before performing the assay.

20S IP Assay Buffer: Bring to room temperature (RT) before use. Store at -20 °C.

20S IP Substrate: Bring to RT before use, to melt the DMSO solution. Divide into aliquots and store at -20 °C, protected from light.

20S IP Positive Control: Divide into aliquots and store at -20 °C. Avoid multiple freeze-thaw cycles. Note: Do not vortex the tube. Pipet up and down to mix and keep on ice when in use.

AMC Standard (1 mM): Bring to RT before use, to melt the DMSO solution. Store at -20 °C, protected from light.

20S IP Inhibitor: Bring to RT before use, to melt the DMSO solution. Store at -20 °C, protected from light.

Sample Preparation

Cells: Add 100 μ l of 20S IP Assay Buffer to 1×10^6 cells and pipette up and down several times on ice. Centrifuge the lysate at 10,000 x *g* and 4 °C for 10 min and collect the clear supernatant for the assay.

Tissues: Transfer ~10 mg of tissue to a Dounce homogenizer (BioVision Cat# 1998) and add 100 μ l of 20S IP Assay Buffer. Homogenize on ice for 5 min to lyse the tissue. Centrifuge the lysate at 10,000 x *g* and 4 °C for 10 min and collect the supernatant for the assay. The lysates can be diluted further, if required, using 20S IP Assay Buffer. Add 1-50 μ l of the sample into two wells labeled as **Sample** and **Inhibitor** respectively. Adjust the volume of the Sample well to 50 μ l with 20S IP Assay Buffer. For the **Inhibitor** well, add 1 μ l of 20S IP Inhibitor and adjust the volume to 50 μ l with 20S IP Assay Buffer. For the **Substrate Background Control** well, add 50 μ l of 20S IP Assay Buffer. For the **Positive Control** well, add 2 μ l of 20S IP Positive Control and adjust the volume to 50 μ l with 20S IP Assay Buffer.

Note: For Unknown Samples, we suggest testing several dilutions to ensure that the readings are within the linear range of the AMC Standard Curve.

Assay Procedure

1. **AMC Standard Curve Preparation**: Dilute the AMC Standard (1 mM) to 10 μ M AMC Standard working solution by adding 10 μ l of 1 mM AMC Standard to 990 μ l of distilled water. Add 0, 2, 4, 6, 8 and 10 μ l of 10 μ M AMC Standard working solution to wells. Adjust the volume of each well to 100 μ l with 20S IP Assay Buffer to generate 0, 20, 40, 60, 80 and 100 pmoles of AMC/well, respectively.
2. **Reaction Mix Preparation**: Mix enough reagents for the number of assays to be performed. For each well prepare a total of 50 μ l Reaction Mix:

	<u>Reaction Mix</u>
20S IP Assay Buffer	48 µl
20S IP Substrate	2 µl

Add 50 µl Reaction Mix to wells containing Sample, Positive Control, Substrate Background Control and Inhibitor and mix well.

Note: Have the microplate reader ready at 37 °C before adding the Reaction Mix to the wells.

3. **Measurement:** Immediately record the fluorescence every 30 sec at Ex/Em = 345/445 nm in kinetic mode at 37 °C for 60 min. AMC Standard Curve can be read in kinetic or end-point mode.

Calculations

Subtract the 0 Standard reading from all Standard readings and plot the AMC Standard Curve. Subtract the **Inhibitor** reading from all Sample readings to get corrected Sample readings. Apply the corrected Sample readings to the AMC Standard Curve to get the value of B pmol of AMC in the sample.

$$\text{Sample 20S Immunoproteasome Activity} = \frac{B \times D}{(\Delta t \times M)} \text{ (pmol / (min} \times \text{mg))} = \mu\text{U/mg}$$

Where: **B** = Amount of AMC in the sample (pmol)

Δt = Reaction time (i.e. **60 min**)

M = Mass of total protein in the sample in the well

D = Dilution factor (D = 1, for undiluted samples)

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information, visit: www.abcam.com/contactus

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