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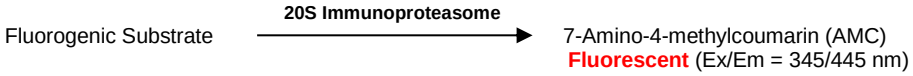
20S Immunoproteasome Activity Assay Kit (Fluorometric)

11/20

(Catalog # K2051-100; 100 assays; Store at -20 ºC)

I. 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 β1, β2 and β5 of the 20S proteasome core are replaced by the inducible subunits β1i/LMP2, β2i/MECL1 and β5i/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 µU (0.02 pmol substrate proteolysis per minute) of 20S immunoproteasome under assay conditions.



II. Application:

- To measure the activity of 20S immunoproteasome in samples.

III. Sample Types:

- Tissue lysate (e.g. spleen tissue)
- Cell lysate
- Purified protein

IV. Kit Contents:

Components	K2051-100	Cap Code	Part Number
20S IP Assay Buffer	25 ml	WM	K2051-100-1
20S IP Substrate	200 µl	Red	K2051-100-2
20S IP Positive Control	20 µl	Green	K2051-100-3
AMC Standard	100 µl	Yellow	K2051-100-4
20S IP Inhibitor	100 µl	Blue	K2051-100-5

V. User Supplied Reagents and Equipment:

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

VI. Storage Conditions and 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.

VII. 20S Immunoproteasome Activity Assay Protocol:

1. Sample Preparation:

Cells: Add 100 µl of 20S IP Assay Buffer to $1X10^6$ 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 µl 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 µl of the sample into two wells labeled as **Sample** and **Inhibitor** respectively. Adjust the volume of the Sample well to 50 µl with 20S IP Assay Buffer. For the **Inhibitor** well, add 1 µl of 20S IP Inhibitor and adjust the volume to 50 µl with 20S IP Assay Buffer. For the **Substrate Background Control** well, add 50 µl of 20S IP Assay Buffer. For the **Positive Control** well, add 2 µl of 20S IP Positive Control and adjust the volume to 50 µl 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.

2. AMC Standard Curve Preparation: Dilute the AMC Standard (1 mM) to 10 µM AMC Standard working solution by adding 10 µl of 1 mM AMC Standard to 990 µl of distilled water. Add 0, 2, 4, 6, 8 and 10 µl of 10 µM AMC Standard working solution to wells. Adjust the volume of each well to 100 µl with 20S IP Assay Buffer to generate 0, 20, 40, 60, 80 and 100 pmoles of AMC/well, respectively.

3. Reaction Mix Preparation: Mix enough reagents for the number of assays to be performed. For each well prepare a total of 50 µl Reaction Mix:

	Reaction Mix
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.

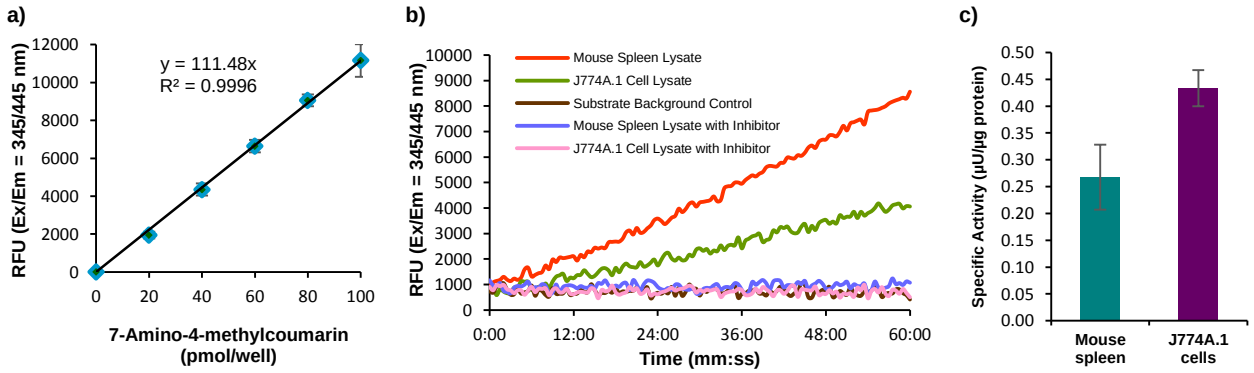
4. **Measurement:** Immediately record the fluorescence every 30 sec at Ex/Em = 345/445 nm in kinetic mode at 37 °C for 60 min. AMC Standard Curve can be read in kinetic or end-point mode.

5. Calculation: 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.

$$\textbf{Sample 20S Immunoproteasome Activity} = \frac{\textbf{B X D}}{\textbf{(}\Delta\textbf{t X M)}}\ \textbf{(pmol / (min X mg)) = µU/mg}$$

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

Unit Definition: One unit of 20S Immunoproteasome is the amount of enzyme that produces 1.0 µmol of AMC per minute at pH 8.0 at 37 °C.



Figures. (a). AMC Standard Curve. **(b).** Enzyme kinetics using mouse spleen lysate (94 µg protein/well) and J774A.1 cell lysate (25 µg protein/well) **(c).** Specific activity of 20S Immunoproteasome in mouse spleen lysate and J774A.1 cell lysate. Experiments were conducted according to the kit protocol.

VIII. Related Products:

Proteasome Activity Fluorometric Assay Kit (K245)
Deubiquitinase Activity Assay Kit (Fluorometric) (K485)
MG-132 (1703)
Lactacystin (Synthetic) (1709)
UCH-L1 Deubiquitinase Inhibitor Screening Kit (K484)
IFN-gamma, human recombinant (4116)
EZSolution™ MG-132 (1791)
Lactacystin (Natural) (2434)

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