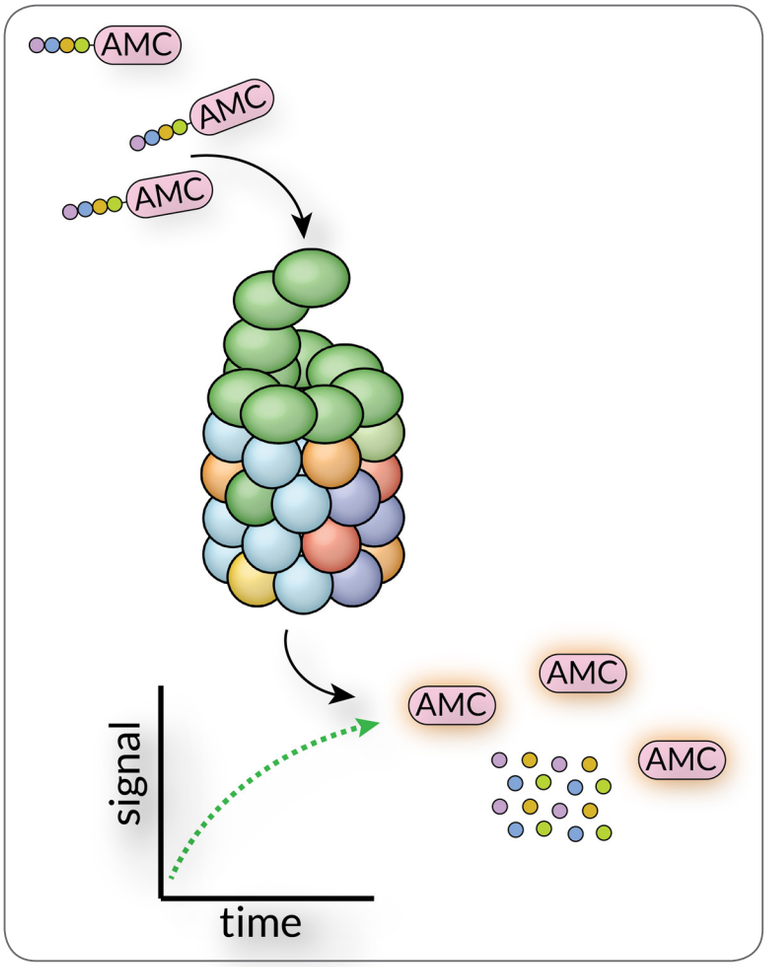
20S Proteasome Kit

Cat. No. SBB-KP0038
Lot. No. 172440038

South Bay Bío

Introduction

This kit is designed to test for specific activity of 20S proteasome. The kit provides purified 20S proteasome and is designed to test for Chymotrypsin-like activity (Suc-LLVY-AMC), Caspase-like activity of the proteasome subunits ß1/PSMB6 (LLE-AMC), and Chymotrypsin-like activity of ß5/PSMB5 (WLA-AMC). Additionally we have included the compound, MG-132, which can be used to inhibit the proteasome. All peptide substrates are conjugated to AMC, which upon proteasome catalyzed hydrolyses display fluorescence at Excitation = 345 nm, Emission = 445 nm; allowing for a real-time read out of 20S proteasome specific activity.



Product Information

Quantity: 100 x 50 µL reactions

Kit Components:

- 10x 20S Proteasome
- 50x LLVY-AMC, Chemotrypsin-like activity
- 50x WLA-AMC, ß5/PSMB5 specific substrate
- 50x LLE-AMC, ß1/PSMB6 specific substrate
- 50x Inhibitor (MG-132, 2mM) in 100% DMSO
- 10x Reaction Buffer
- 50x SDS(1.75%) in H20
- 100x free AMC Standard(40 uM)

Storage: -80C, Avoid multiple freeze / thaw cycles. It is recommended to make aliquots of each reaction component upon first time use.

Setup Protocol

1) It is recommended to make 2 solutions (A & B), and initiate the kinetic reaction by mixing them together in equal proportions immediately before reading.

2) Mix components in this order for Solutions A & B:
Example setup for <u>1 mL</u> final reaction volume mix (20 wells x 50uL):

Solution A (500µL)	Solution B (500µL)
420µL of H20	420µL H20
50µL of 10x Reaction Buffer	50µL 10x of Reaction Buffer
10µL of 50x SDS	10µL of 50x SDS
20µL 50x Proteasome	**20µL 50x AMC Substrate**

Place 25µL of Solution A into each well, and initiate reaction with addition of 25µL of Solution B (containing your choice of either LLVY-AMC / WLA-AMC / LLE-AMC).

Optional: Add 1.0µL of 50x inhibitor to negative control wells before reaction initiation to inhibit Proteasome substrate hydrolysis. If electing to use inhibitor be sure to add 1.0µL DMSO (not supplied) to all sample wells to match final DMSO concentration.

3) Read top-read black/opaque half-well plates at Excitation = 345 nm, Emission = 445 nm in kinetic mode.

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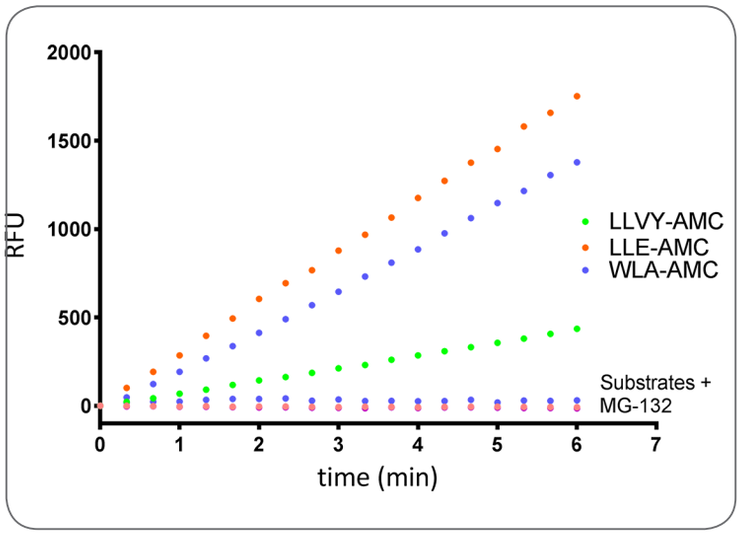
Raw Data Output: Endpoint & Kinetic

Raw data output is usually in relative fluorescence units (rfu). During a kinetic read you will observe the formation of product signal (free AMC) in rfu over time, i.e. a rate. An example of a typical substrate-AMC digestion reaction is shown in the scheme and figure below:

Substrate [LLVY-AMC]* + i20S → Product [AMC]** + i20S



Raw Data Output: Several wells of Proteasome shown digesting LLVY, WLA, and LLE-AMC over time +/- 1x (40uM) inhibitor (MG-132).

Data Reduction & Standard Curve

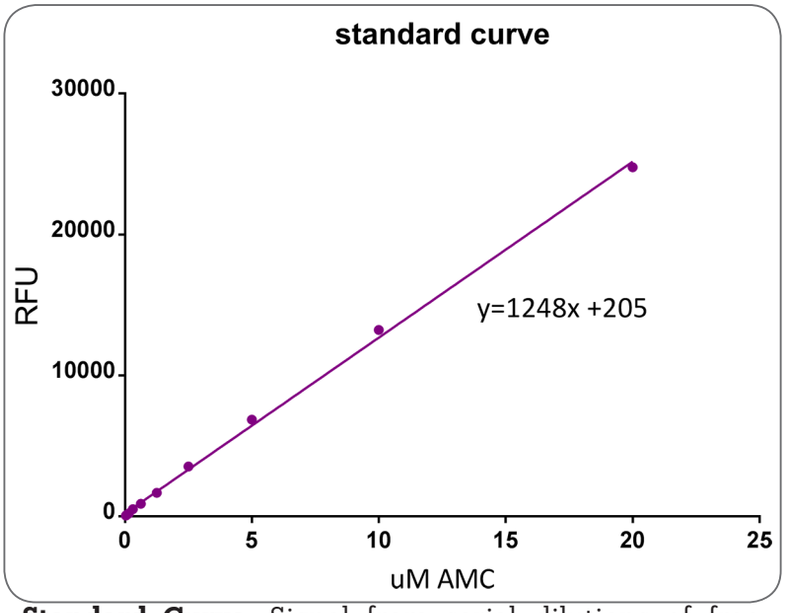
To quantify rates into meaningful units beyond rfu (s^{-1}) a standard curve must be generated. This kit supplies free AMC standard at 40µM, or 100x the concentration of the recommended standard curves highest concentration.

Example protocol for Standard Curve generation:

1) Prepare 1x stock of free AMC standard at 0.4µM using 1x Reaction buffer.

2) Make 2x serial dilutions of 1x AMC standard (from 0.4µM to 0.0125µM). Add 50uL of each serial dilution to black/opaque half-well plates and read at Excitation = 345 nm, Emission = 445 nm in plate reader.

3) Plot signal (rfu) vs. AMC standard concentration in µM (x-axis), and fit a linear regression curve to the data as shown below. The slope of the regression line corresponds to rfu/µM AMC standard:



Standard Curve: Signal from serial dilutions of free AMC standard is used to acquire a conversion factor corresponding to the slope of the regression line fit to the data, in units of rfu/µM AMC standard. In this example 1248rfu/µM AMC).

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Data Reduction & Standard Curve-Cont.

4) Divide your initial velocity rates rfu (s-1) from your experiment by the slope of your standard curve's regression line to convert rates to uM AMC (s-1).

$$\frac{\cancel{\text{rfu}}}{\text{second}} \bullet \frac{\mu\text{M AMC}}{\cancel{\text{rfu}}} = \frac{\mu\text{M AMC}}{\text{second}}$$

References

1) Smith DM, Chang SC, Park S, Finley D, Cheng Y, Goldberg AL (Sep 2007). "Docking of the proteasomal ATPases' carboxyl termini in the 20S proteasome's alpha ring opens the gate for substrate entry". Molecular Cell. 27 (5): 731–44. doi:10.1016/j.molcel.2007.06.033. PMC 2083707Freely accessible. PMID 17803938.

2) Wilk S, Orlowski M (Mar 1983). "Evidence that pituitary cation-sensitive neutral endopeptidase is a multicatalytic protease complex". Journal of Neurochemistry. 40 (3): 842–9. doi:10.1111/j.1471-4159.1983.tb08056.x. PMID 6338156.

3) Kisselev, Alexei F., and Alfred L. Goldberg. "Monitoring activity and inhibition of 26S proteasomes with fluorogenic peptide substrates." Methods in enzymology 398 (2005): 364-378.

4) Ettari, Roberta, et al. "Immunoproteasome-selective inhibitors: a promising strategy to treat hematologic malignancies, autoimmune and inflammatory diseases." Current medicinal chemistry 23.12 (2016): 1217-1238.

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