

ab107921

Proteasome Activity Assay Kit (Fluorometric)

Instructions for Use

For the rapid, sensitive and accurate measurement of proteasome-specific activity in various samples.

[View kit datasheet: www.abcam.com/ab107921](http://www.abcam.com/ab107921)

(use www.abcam.cn/ab107921 for China, or www.abcam.co.jp/ab107921 for Japan)

This product is for research use only and is not intended for diagnostic use.

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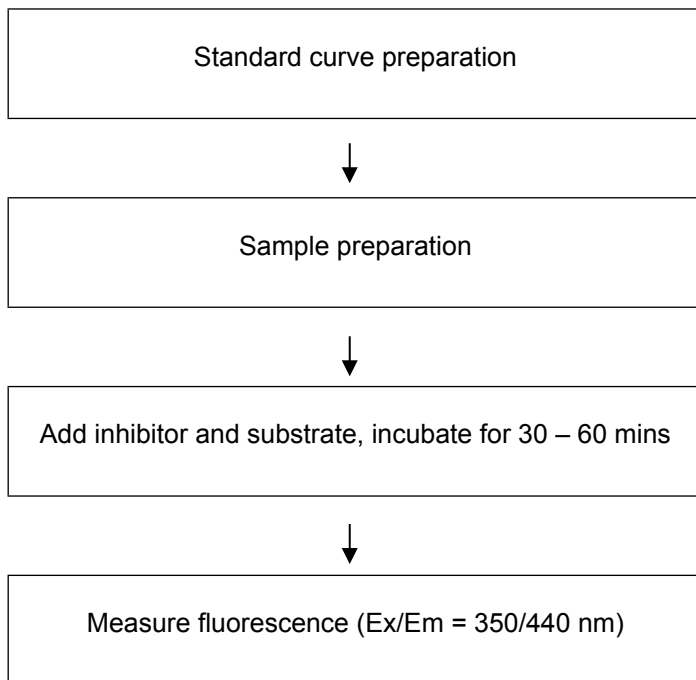
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1. BACKGROUND

Abcam's Proteasome Activity Assay Kit (Fluorometric) (ab107921) 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.

Proteasomes are very large (20S, 26S) protein assemblies found in both the nucleus and cytoplasm of all eukaryotes (and in some prokaryotes). 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.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

5. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Proteasome Assay Buffer	25 mL	-20°C	-20°C
Proteasome Substrate	0.1 mL	-20°C	-20°C
Proteasome Inhibitor	100 µL	-20°C	-20°C
AMC Standard	100 µL	-20°C	-20°C
Proteasome Positive Control	1 vial	-20°C	-80°C

PLEASE NOTE: Proteasome Positive Control was previously labelled as Positive Control III and Positive Control (Lyophilized). The composition has not changed.

7. MATERIALS REQUIRED, NOT SUPPLIED

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

- MilliQ water or other type of double distilled water (ddH₂O)
- PBS
- Fluorescent microplate reader – equipped with filter Ex/Em = 350/440 nm
- 96 well plate (opaque white well plate recommended for this assay)
- Microcentrifuge
- Pipettes and pipette tips
- Heat block or water bath
- Vortex
- NP-40

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Keep enzymes, heat labile components and samples on ice during the assay.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **Proteasome Assay Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 **Proteasome Substrate:**

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use.

NOTE: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for few minutes at 37°C. Store at -20°C protected from light.

9.3 **Proteasome Inhibitor:**

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use.

NOTE: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for few minutes at 37°C. Store at -20°C protected from light.

9.4 **AMC Standard:**

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use.

NOTE: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for few minutes at 37°C. Store at -20°C protected from light.

9.5 **Proteasome 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 °C. Keep on ice while in use. Avoid repeated freeze/thaw cycles.

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and should not be stored for future use.

10.1 Prepare 0.01 mM AMC Standard by diluting 10 μL of 1 mM AMC Standard to 990 μL of ddH₂O.

10.2 Using 0.01 mM AMC Standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of Standard (μL)	Assay Buffer (μL)	Final volume standard in well (μL)	End [AMC] in well
1	0	300	100	0 pmol/well
2	6	294	100	20 pmol/well
3	12	288	100	40 pmol/well
4	18	282	100	60 pmol/well
5	24	276	100	80 pmol/well
6	30	270	100	100 pmol/well

Each dilution has enough amount of standard to set up duplicate readings (2 x 100 μL).

NOTE: *If your sample readings fall out the range of your fluorometric standard curve, you might need to adjust the dilutions and create a new standard curve.*

11. SAMPLE PREPARATION

General Sample information:

- This product detects proteolytic activity. Do not use protease inhibitors in the sample preparation step as it might interfere with the assay.
- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C . When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 **Cell (adherent or suspension) samples:**

- 11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation = 2×10^6 cells).
- 11.1.2 Wash cells with cold PBS.
- 11.1.3 Resuspend cells in 0.5% NP-40 in dH_2O or PBS (~4 volumes).
- 11.1.4 Homogenize cells quickly by pipetting up and down a few times.
- 11.1.5 Centrifuge 10 – 15 minutes at 4°C at 13,000 rpm using a cold microcentrifuge to remove any insoluble material.
- 11.1.6 Collect supernatant and transfer to a clean tube.

NOTE: Do not use the protease inhibitors during cell lysate preparation.

NOTE: We suggest using different volumes of sample to ensure readings are within the Standard Curve range.

12. ASSAY PROCEDURE and DETECTION

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend to assay all standards, controls and samples in duplicate.
- All samples and the positive control should be assayed with and without proteasome inhibitor.

12.1 Set up Reaction wells:

- Standard wells = 100 μ L standard dilutions.
- Sample wells = 1 – 50 μ L samples in duplicate (adjust volume to 100 μ L/well with Assay Buffer).
- Positive Control wells = 10 μ L Proteasome Positive Control in duplicate (adjust volume to 100 μ L/well with Assay Buffer).
- Background wells = 100 μ L Assay Buffer.

12.2 Add Proteasome Inhibitor or Assay Buffer to a pair of positive control wells and each pair of sample wells as shown below and mix well:

Component	Standard wells	Positive control wells		Sample wells	
		With inhibitor (μ L)	Without inhibitor (μ L)	With inhibitor (μ L)	Without inhibitor (μ L)
Proteasome inhibitor*	0	1	0	1	0
Assay buffer	0	0	1	0	1

**Proteasome inhibitor needs to be optimized for each sample type. 1 μ L of inhibitor achieves about 50% inhibition of positive control.*

- 12.3 Add 1 μ L of Proteasome substrate to all sample and positive control wells protected from light.
- 12.4 Mix well. Incubate at 37°C protected from light.
- 12.5 Measure output on a fluorometric microplate reader at T₁ at Ex/Em = 350/440 nm.

NOTE: *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°C. It is essential to read RFU_1 , $iRFU_1$ (at T_1), RFU_2 and $iRFU_2$ (at T_2) in the linear reaction range. It will be more accurate if you monitor the reaction kinetics and then choose T_1 and T_2 in the appropriate linear range. From our experience, initial readings RFU_1 and $iRFU_1$ should be measured after ~ 20-25 min (T_1).*

- 12.6 Incubate at 37°C for 30 minutes (longer if sample activity is low) protected from light.
- 12.7 Measure output on a fluorometric microplate reader at T_2 at Ex/Em = 350/440 nm.

NOTE: *Measurement of the wells which do not contain Proteasome Inhibitor will show total proteolytic activity RFU and the wells containing Proteasome Inhibitor will show non-proteasome activity $iRFU$.*

13. CALCULATIONS

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
 - 13.1 Average the duplicate reading for each standard and sample.
 - 13.2 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.
 - 13.3 Plot the corrected absorbance values for each standard as a function of the final concentration of AMC.
 - 13.4 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).
 - 13.5 The RFU generated by proteasome activity is:

$$\Delta\text{RFU} = (\text{RFU}_2 - i\text{RFU}_2) - (\text{RFU}_1 - i\text{RFU}_1).$$

NOTE: *Measurement of the wells which do not contain Proteasome Inhibitor will show total proteolytic activity RFU and the wells containing Proteasome Inhibitor will show non-proteasome activity iRFU.*
 - 13.6 Apply the ΔRFU to the AMC standard curve to get B pmol of AMC (amount generated between T_1 and T_2 in the reaction wells specifically by proteasome activity).

13.7 Activity of proteasome in the test samples is calculated as:

$$\text{Proteasome Activity} = \left(\frac{B}{(T_2 - T_1) * V} \right) * D$$

Where:

B = Amount of AMC in the sample well (pmol).

V = Sample volume added into the reaction well (μL).

T_1 = Time (min) of the first reading (RFU_1 and iRFU_1).

T_2 = Time (min) of the first reading (RFU_2 and iRFU_2).

D = Sample dilution factor.

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

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

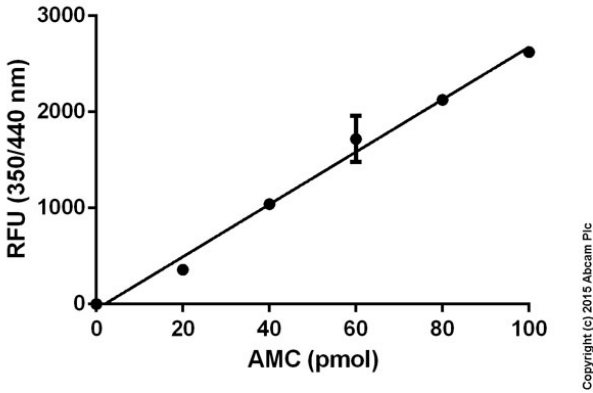


Figure 1. Standard curve: mean of duplicates (+/- SD) with background reads subtracted.

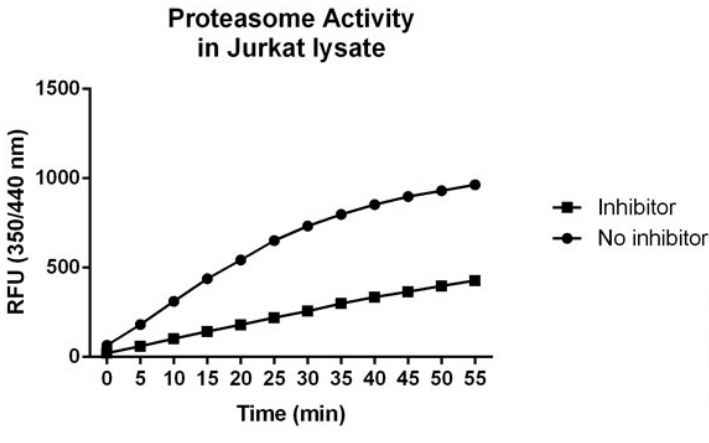
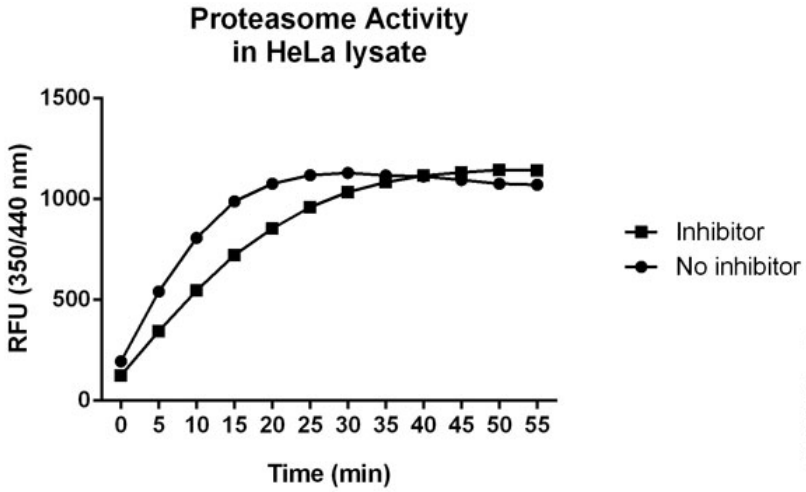


Figure 2: Proteasome Activity measured in Jurkat lysate.



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Figure 3: Proteasome Activity measured in HeLa lysate.

15. QUICK ASSAY PROCEDURE

NOTE: *This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Prepare AMC Standard, Proteasome Positive Control, Proteasome Substrate and Inhibitor (aliquot if necessary); get equipment ready.
- Prepare standard curve.
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Set up plate for standard (100 μ L), samples (100 μ L), positive control (100 μ L) and background wells (100 μ L).
- Add 1 μ L of Proteasome Inhibitor to one of the sample and positive control duplicate wells and mix.
- Add 1 μ L of Assay Buffer to the other sample and positive control well and mix.
- Add 1 μ L of Proteasome Substrate to all sample and control wells and mix.
- Incubate at 37°C protected from light.
- Measure T₁ output (Ex/Em = 350/440 nm) on a microplate reader.
- Incubate at 37°C for 30 mins (longer if sample activity is low) protected from light.
- Measure T₂ output (Ex/Em = 350/440 nm) on a microplate reader.

16. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: white opaque wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

RESOURCES

Problem	Cause	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 μL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17. FAQ

18. INTERFERENCES

These chemicals or biological materials will cause interferences in this assay causing compromised results or complete failure.

- RIPA: contains SDS which can destroy/decrease the activity of the enzyme
- Protease inhibitors.

19. NOTES

Technical Support

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