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ab273291 Acidic Mammalian Chitinase Activity Kit (Fluorometric)

View Kit datasheet: https://www.abcam.com/ab273291
(use https://www.abcam.cn/ab273291 for china, or
https://www.abcam.co.jp/ab273291 for Japan)

For the determination of AMCase activity in cell and tissue lysates and of purified AMCase enzyme.

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

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Table of Contents

1. Overview

Acidic Mammalian Chitinase Activity Kit (Fluorometric) (ab273291) utilizes the ability of AMCase to cleave a synthetic substrate to release the free fluorophore which can be easily quantified (Ex/Em= 320/445 nm) and it uses a specific Lysis Buffer which distinguishes AMCase activity from CHIT1 activity. This kit provides a simple, specific, ultra-sensitive assay that can detect as low as 0.5 mU/ml of AMCase activity in a variety of biological samples.

2. Protocol Summary

Prepare lysates as directed

Prepare all reagents as directed

Prepare standard curve and measure fluorescence at Ex/Em = 320/445nm in end-point mode

Add Positive Control, Samples and Sample Background Controls to appropriate wells and adjust volume to 50 µL

Add Substrate Mix (50 µL) to Positive Control and Samples. Add Assay Buffer LI/AMCase Assay buffer (50 µL) containing preotease inhibitors to Sample Background Control

Measure fluorescence (Ex/Em = 320/445 nm) in kinetic mode for 20-30 minutes at 37°C.

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.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt.

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.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- 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	Quantity	Storage temperature (before prep)
Assay Buffer LI/AMCase Assay Buffer	25 mL	-20°C or +4°C
CHIT1 Inhibition Buffer/AMCase Lysis Buffer	18 mL	-20°C or +4°C
Chitinase Substrate/AMCase Substrate	25 µL	-20°C
AMCase Positive Control/Acidic Mammalian Chitinase (lyophilized)	1 vial	-20°C
4-Methylumbelliferone Standard (5 mM)	35 µL	-20°C

7. Materials Required, Not Supplied

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

- Fluorescence microplate reader capable of measuring fluorescence at Ex/Em = 320/445 nm
- 96-well white opaque plate

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.
- 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.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 **Assay Buffer LI/AMCase Assay Buffer:**

Store at -20 °C or 4 °C. Bring to 37 °C before use.

9.2 **CHIT1 Inhibition Buffer/AMCase Lysis Buffer:**

Store at -20 °C or 4 °C. Keep on ice while in use.

9.3 **Chitinase Substrate/AMCase Substrate:**

Ready to use. Store at -20°C. Bring to room temperature before use.

9.4 **AMCase Positive Control/Acidic Mammalian Chitinase (Lyophilized):**

Reconstitute AMCase Positive Control/Acidic Mammalian Chitinase in 100 µL Assay Buffer LI/AMCase Assay Buffer and mix thoroughly. Aliquot and store at -20°C. Avoid repeated freeze/thaw. Keep on ice while in use. Use within 2 months.

9.5 **4-Methylumbelliferone Standard (5 mM):**

Store at -20°C. Bring to room temperature before use. Light sensitive.

10. Sample Preparation

10.1 Tissue/cell lysate preparation:

10.1.1 Homogenize cells (~1 x 10^6) or tissue (5-20 mg) with 150 µL of iced-cold CHIT1 Inhibition Buffer/AMCase Lysis Buffer containing protease inhibitor cocktail and keep on ice for 10 min.

10.1.2 Centrifuge samples at 12,000 x *g* at 4 °C for 5 min. and collect the supernatant.

Δ Note: Measure AMCase activity in samples on the day of sample preparation. Do not store lysed samples.

11. Standard Curve

11.1 Prepare a 100 µM solution of 4-MU Standard by diluting 5 µL of 5 mM 4-MU-Standard with 245 µL of Assay Buffer LI/AMCase Assay Buffer.

11.2 Further dilute the 100 µM standard solution by adding 20 µL of 100 µM 4-MU to 180 µL Assay Buffer LI/AMCase Assay Buffer to generate a 10 µM standard.

11.3 Add 0, 10, 20, 30 and 40 µL of 10 µM 4-MU-Standard into a series of wells in a 96-well plate and adjust the final volume to 100 µL/well with Assay Buffer LI/AMCase Assay Buffer. This will generate 0, 100, 200, 300, 400 pmol/well of 4-MU Standard respectively.

Standard #	10 µM 4-MU-Standard (µL)	Assay Buffer LI/AMCase Assay Buffer (µL)	4-MU (pmol/well)
1	40	60	400
2	30	70	300
3	20	80	200
4	10	90	100
5	0	100	0

11.4 Mix well and measure the fluorescence (Ex/Em = 320/445 nm) in an end point mode.

Δ Note: Equilibrate the Standard Solution to 37 °C before adding to the wells.

12. Assay Procedure

Thaw all reagents thoroughly and mix gently.

12.1.1 For Sample and Sample Background Control , add 2-20 µL of lysate into desired well(s) in a 96-well plate.

Δ Note: For unknown samples, we recommend doing pilot experiment and testing several doses to ensure the readings are within the Standard Curve range. Do not use more than 20 µl of sample in each well.

12.1.2 For Positive Control, add 4-8 µL of the diluted AMCase Positive Control into desired well(s).

12.1.3 Adjust the volume of Sample, Sample Background Control and Positive Control to 50 µL/well with Assay Buffer LI/AMCase Assay Buffer. Mix well.

12.1.4 Incubate for 15 min. at room temperature.

MCA substrate mix:

12.1.5 Prepare a 225-fold dilution of Chitinase Substrate/AMCase substrate stock solution as shown in the table.

12.1.6 Vortex briefly and keep in ice.

Component	Volume to add per well
Assay Buffer LI/AMCase Assay Buffer	448 µl
Chitinase Substrate/AMCase Substrate	2 µl

Δ Note: Equilibrate the Substrate Solutions to 37 °C before adding to the wells.

12.1.7 Add 50 µL of Diluted Chitinase/AMCase Substrate Mix to Sample and Positive Control wells. Mix well.
12.1.8 For Sample Background Control, add 50 µL Assay Buffer LI/AMCase Assay Buffer containing protease inhibitor cocktail/
12.1.9 Measure fluorescence (Ex/Em = 320/445 nm) in kinetic mode for 20-30 minutes at 37 °C.

Δ Note: Incubation time depends on the AMCase activity in samples. Longer incubation time may be required for samples having low AMCase activity.

13. Calculations

13.1 Choose two time points (t_1 & t_2) in the linear range of the plot and obtain the corresponding values for the fluorescence (RFU_1 and RFU_2).
13.2 Measure fluorescence intensity of Standard Solution (see Section 11) at Ex/Em= 320/445 nm at 37 °C with end point setting using a fluorescence microtiter plate reader.
13.3 Subtract 0 Standard reading from all readings.
13.4 Plot the MCA-Standard Curve to obtain the corresponding amount of 4-MU formed.
13.5 Calculate the background-corrected sample ΔRFU (B, in pmol) by subtracting the amount of 4-MU formed by Sample Background Control from the amount of 4-MU formed by Sample and calculate the activity of AMCase activity in the sample as:

$$Sample\ AMCase\ activity\ = \frac{B}{(\Delta t\ x\ V)} X\ D \quad (pmol/min/ml)$$

B = 4-MU produced in Samples based on the Standard Curve (pmol)
Δt = Reaction time ($t_2 – t_1$) in minutes
V = Sample volume used (ml)
D = Sample dilution factor

AMCase activity can be expressed in U/mg of protein

Unit definition:

One unit of AMCase activity is the amount of enzyme that generate 1.0 µmol of 4-MU per min., at pH 4.2 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.

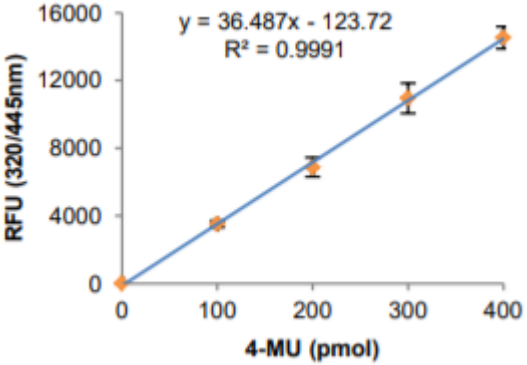

Figure 1. 4-MU Standard Curve. Results from multiple experiments.

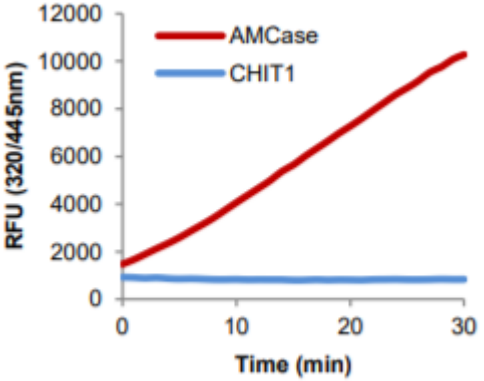

Figure 2. Measurement of purified AMCase (2 ng) and CHIT1 (2 ng) activities. The kit can effectively discriminate AMCase activity from CHIT1 activity using our proprietary CHIT1 Inhibition Buffer/Lysis Buffer

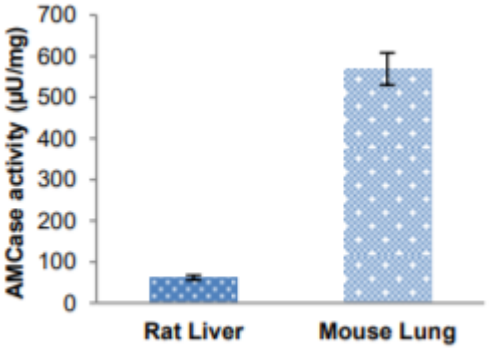

Figure 3. Measurement of AMCase activity in Rat Liver (20 µg protein) and Mouse Lung (2.5 µg protein). All assays were performed following kit protocol.

15. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

16. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)