



Acidic Mammalian Chitinase Activity Kit (Fluorometric)

06/16

(Catalog # K513-100; 100 assays; Store at -20°C)

I. Introduction:

Acidic Mammalian Chitinase (AMCase) and Chitotriosidase-1(CHIT1) are the only two mammalian active chitinases that belong to the glycosyl hydrolase-18 family (EC 3.2.1.14). They are chitin-fragmenting hydrolases that hydrolyze the β -(1,4)-linkage between the adjacent N-acetyl glucosamine residues of chitin. In contrast to CHIT1, AMCase exhibits its optimum activity under acidic conditions and is relatively abundant in the gastrointestinal tract and lungs. AMCase is found highly expressed both in animal models used for the study of allergic inflammation and in lungs of human asthma. Recent data suggests a major biological function of AMCase in asthma and the changes in AMCase expression can be used to monitor Th-2/IL-13-mediated immune response and inflammation. BioVision's Acidic Mammalian Chitinase Activity Assay Kit 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.



II. Applications:

- Tissue homogenates: lung, liver, etc.
- Cell culture: adherent or suspension cells
- Purified enzyme

III. Kit Contents:

Components	K513-100	Cap Code	Part Number
AMCase Assay Buffer	25 ml	WM	K513-100-1
AMCase Lysis Buffer	18 ml	NM	K513-100-2
AMCase Substrate (in DMSO)	25 μ l	Red	K513-100-3
Acidic Mammalian Chitinase (lyophilized)	1 vial	Green	K513-100-4
4-Methylumbelliferone Standard (5 mM)	35 μ l	Yellow	K513-100-5

IV. User Supplied Reagents and Equipment:

- 96-well white opaque plate
- Multi-well spectrophotometer (fluorescence plate reader)

V. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **AMCase Assay Buffer:** Warm to 37 °C before use. Store at either 4°C or -20°C.
- **AMCase Lysis Buffer:** Keep on ice while in use. Store at either 4°C or -20°C.
- **AMCase substrate:** Store at -20°C. Bring to room temperature before use.
- **Acidic Mammalian Chitinase:** Reconstitute Acidic Mammalian Chitinase in 100 μ l AMCase Assay Buffer and mix thoroughly. Aliquot and store at -20°C. Avoid repeated freeze/thaw. Keep on ice while in use. Use within two months.
- **4-Methylumbelliferone Standard:** Light sensitive. Store at -20°C. Bring to room temperature before use.

VI. Acidic Mammalian Chitinase Assay Protocol:

1. Sample Preparation: Homogenize cells (~1x10⁶) or tissue (5-20 mg) with 150 μ l of iced-cold AMCase Lysis Buffer containing protease inhibitor cocktail (Cat. # K272 or equivalent) and keep on ice for 10 min. Centrifuge samples at 12,000 x g at 4 °C for 5 min. and collect the supernatant. Add 2-20 μ l of sample into desired well(s) in a white 96-well plate labeled as Sample and Sample Background Control. For positive control, add 4-8 μ l of Acidic Mammalian Chitinase into desired well(s). Adjust the volume of Positive Control, Sample Background Control and Sample wells to 50 μ l/well with AMCase Assay Buffer.

Note:

- a. 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 μ l of sample in each well.
- b. Measure AMCase activity in samples on the day of sample preparation. Do not store lysed samples.

2. Standard Curve Preparation: Prepare a 100 μ M 4-Methylumbelliferone (4-MU) by adding 5 μ l of 5 mM 4-MU to 245 μ l AMCase Assay Buffer. Further dilute the 100 μ M Standard Solution by adding 20 μ l of 100 μ M to 180 μ l AMCase Assay Buffer to generate 10 μ M 4-MU Standard. Add 0, 10, 20, 30, 40 μ l of 10 μ M 4-MU standard into a series of wells to generate 0, 100, 200, 300, 400 pmol of 4-MU/well respectively. Adjust the volume to 100 μ l/well with AMCase Assay Buffer.

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

3. AMCase Substrate Solution Preparation: Prepare a 225-fold dilution of AMCase Substrate Stock Solution (i.e. Dilute 2 μ l of AMCase Substrate with 448 μ l of AMCase Assay Buffer), vortex briefly and keep in ice. Add 50 μ l of Diluted AMCase Substrate Solution to each well containing test sample(s) labeled as Sample, and AMCase positive control(s). For Sample Background Control, add 50 μ l of AMCase Assay Buffer containing protease inhibitor cocktail.

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

4. Measurement: Measure fluorescence (Ex/Em= 320/445nm) in kinetic mode at 37 °C for 20-30 min. Choose two time points (t_1 and t_2) in the linear range of the plot and obtain the corresponding fluorescence values (RFU₁ and RFU₂). Measure fluorescence intensity of Standard Solution (see step 2.) at Ex/Em= 320/445 nm at 37 °C with end point setting using a fluorescence microtiter plate reader.

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

5. Calculation: Subtract 0 Standard reading from all standard readings. Plot the 4-MU Standard Curve; apply Sample Δ RFU and Sample Background Control Δ RFU to 4-MU Standard Curve to obtain the corresponding amount of 4-MU formed. Calculate the background-corrected sample Δ RFU (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:

$$\text{Sample AMCase} = \frac{B}{(\Delta t \times V)} \times D = \text{pmol/min/ml} = \mu\text{U/ml}$$

Where: **B** = 4-MU from Standard Curve (pmol)

Δt = Reaction time (min.)

V = Sample volume added into the reaction well (ml)

D = Dilution Factor

AMCase specific activity can be expressed as U/mg of protein.

Unit Definition: One unit of AMCase activity is the amount of enzyme that generate 1.0 μ mol of 4-MU per min., at pH 4.2 at 37 °C.

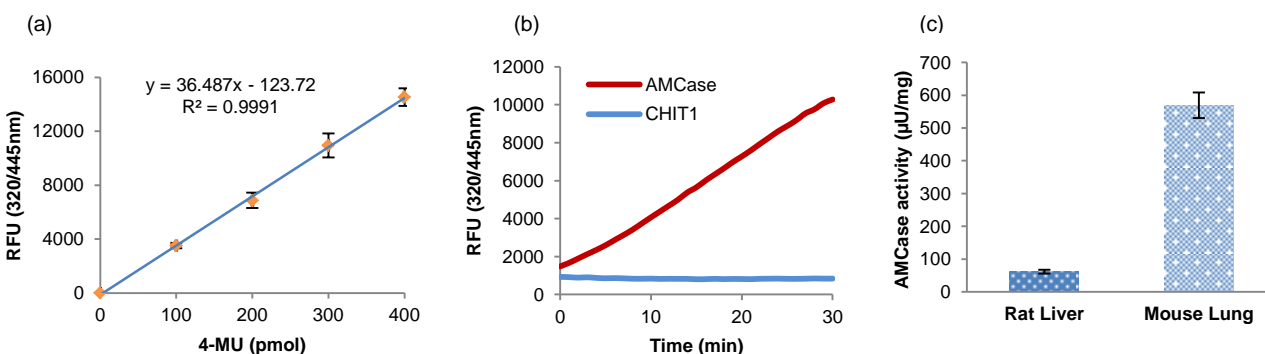


Figure: (a) 4-Methylumbelliferon Standard Curve, results from multiple experiments. (b) Measurement of purified AMCase (2 ng) and CHIT1 (2 ng) activities. The kit can effectively discriminate AMCase activity from CHIT1 activity using our proprietary Lysis Buffer. (c) Measurement of AMCase activity in Rat Liver (20 μ g protein) and Mouse Lung (2.5 μ g protein). All assays were performed following kit protocol.

VII. RELATED PRODUCTS:

Lysozyme Activity Assay Kit (K236)

Lysozyme Inhibitor Screening Kit (K237)

Acidic Mammalian Chitinase Inhibitor Screening Kit (693)

Chitotriosidase Activity Kit (K512)

FOR RESEARCH USE ONLY! Not to be used on humans.