



Acidic Mammalian Chitinase Inhibitor Screening Kit (Fluorometric)

(Catalog # K693-100; 100 assays; Store at -20°C)

I. Introduction:

Acidic Mammalian Chitinase (AMCase) belongs to the glycosyl hydrolase-18 family (EC 3.2.1.14), which is capable of hydrolyzing the β -(1, 4)-linkage between the adjacent N-acetyl glucosamine residues of chitin. AMCase is relatively abundant in the gastrointestinal tract and lung. It is highly expressed in the lungs of asthmatic patients and asthma animal models. In addition, the change in AMCase expression can be used to monitor Th-2/IL-13 mediated immune response and inflammation. Inhibition of AMCase activity has been considered as a therapeutic strategy to alleviate symptoms of allergic airway inflammation. Thus, there is a need to identify AMCase inhibitors as potential candidates to treat and/or prevent asthma. Biovision's Acidic Mammalian Chitinase Inhibitor Screening Kit uses Bisdionin F— a highly selective AMCase inhibitor as positive control and it provides a simple, rapid, sensitive and reliable test suitable for screening/characterizing AMCase inhibitors.

Cleaved substrate + Fluorescent product (Ex/Em=320/445nm)

AMCase substrate

Bisdionin F

II. Applications:

• Screening/characterizing Acidic Mammalian Chitinase inhibitors

III. Kit Contents:

Components	K693-100	Cap Code	Part Number
AMCase Assay Buffer	25 ml	WM	K693-100-1
AMCase Substrate (in DMSO)	25 µl	Red	K693-100-2
Acidic Mammalian Chitinase (lyophilized)	1 vial	Green	K693-100-3
Bisdionin F (5 mM)	20 µl	Brown	K693-100-4

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 Substrate: Store at -20°C. Bring to room temperature before use.
- Acidic Mammalian Chitinase: Reconstitute Acidic Mammalian Chitinase in 550 µl 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.
- Bisdionin F: Store at -20°C. Bring to room temperature before use.

VI. Acidic Mammalian Chitinase Inhibitor Screening Protocol:

1. Screening compounds, inhibitor Control & Blank Control preparations: Dissolve test sample to 100X in a proper solvent. Further dilute to 10X in AMCase Assay Buffer. Prepare a 20-fold dilution of Bisdionin F (i.e. Add 2 µl of the 5 mM Bisdionin F stock solution to 38 µl AMCase Assay Buffer and mix thoroughly. Add 10 µl diluted test sample or Diluted Bisdionin F or AMCase Assay Buffer into wells assigned as test sample (sample, S), Inhibitor Control (IC), or AMCase Enzyme Control (EC) wells, respectively. Additional wells with serial dilutions of the test sample may be prepared at this time if desired, containing 10 µl in each candidate well.

	[S]	[EC]	[IC]
Test Inhibitor	10 µl	-	-
Bisdionin F	-	-	10 µl
AMCase Assay Buffer	-	10 µl	-

Note:

Various solvents, in which certain inhibitors are dissolved in, can reduce the AMCase enzyme activity. Prepare parallel well(s) as Solvent Control (SC) to test the effect of the solvent on AMCase activity. In case SC is significantly different from EC use its values to determine effect of tested compound.

- 2. AMCase Enzyme Solution Preparation: Prepare a 16-fold dilution of Acidic Mammalian Chitinase (i.e. Dilute of 20 μl of Acidic Mammalian Chitinase with 300 μl of AMCase Assay Buffer), mix thoroughly and keep on ice. Add 80 μl of Diluted AMCase Enzyme Solution to each well containing test sample, Inhibitor Control, Solvent Control and AMCase Enzyme Control. Mix well and incubate at 37 °C for 10-15 min. Protect from light.
- **3. AMCase Substrate Solution Preparation:** Prepare a 45-fold dilution of AMCase Substrate Stock Solution (i.e. Dilute 1 μl of AMCase Substrate with 44 μl of AMCase Assay Buffer), vortex briefly and keep in ice. After incubation time (step 2), add 10 μl of Diluted AMCase Substrate Solution to each well containing test sample, Inhibitor Control, Solvent Control and AMCase Enzyme Control.
- **4. Measurement:** Measure fluorescence (Ex/Em= 320/445nm) in kinetic mode at 37 °C for 30 min. Choose two points (t₁ and t₂) in the linear range of the plot and obtain the corresponding fluorescence values (RFU₁ and RFU₂).
- 5. Calculation: Calculate the slope for all samples, including Enzyme Control (EC), by dividing the net △RFU (RFU₂-RFU₁) values by the time △t (t₂-t₁). Calculate % Relative Inhibitor and % Relative Activity as follows:





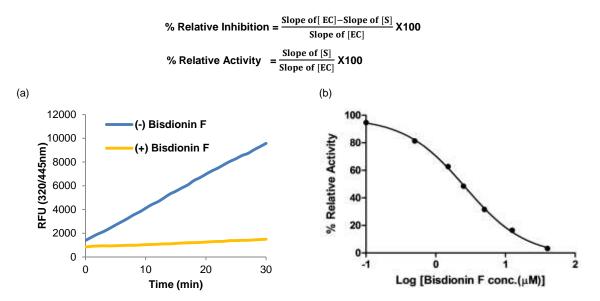


Figure: (a) Progress curve of Acidic Mammalian Chitinase activity in the presence or absence of the inhibitor Bisdionin F. (b) IC_{50} of Bisdionin F was calculated to be 2.666 μ M. Assay was carried out following the kit protocol.

VII. RELATED PRODUCTS:

Lysozyme Activity Assay Kit (K236) Lysozyme Inhibitor Screening Kit (K237)

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