ab283405 – AMCase Inhibitor Screening Kit (Fluorometric)

For the screening of potential AMCASE inhibitors.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab283405

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Upon opening, use kit within 6 months.

Materials Supplied

Item	Quantity	Storage Condition
AMCase Assay Buffer	25 mL	-20°C
Acidic Mammalian Chitinase (lyophilized)	1 vial	-20°C
AMCase Substrate (in DMSO)	25 µl	-20°C
Bisdionin F (5 mM)	20 µl	-20°C

Materials Required, Not Supplied

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

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

Reagent Preparation

- Briefly centrifuge small vials prior to opening.

AMCase Assay Buffer: Warm to 37 °C before use. Store at either 4°C or -20°C.

AMCase Substrate: Warm to room temperature (RT) before use.

Acidic Mammalian Chitinase (lyophilized): 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 (5 mM): Warm to room temperature (RT) before use.

Assay Protocol

- **Test Compound preparation:**

1. Dissolve test sample to 100X in a proper solvent.
2. Further dilute to 10X in AMCase Assay Buffer.
3. 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.
4. 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.
5. Additional wells with serial dilutions of the test sample may be prepared at this time if desired, containing 10 µl in each candidate well.

Δ 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.*

- **AMCase Enzyme Solution Preparation:**

1. 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.
2. Add 80 µl of Diluted AMCase Enzyme Solution to each well containing test sample, Inhibitor Control, Solvent Control and AMCase Enzyme Control.
3. Mix well and incubate at 37 °C for 10-15 minutes. Protect from light.

- **AMCase Substrate Solution Preparation:**

1. 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.
2. After incubation time add 10 µl of Diluted AMCase Substrate Solution to each well containing test sample, Inhibitor Control, Solvent Control and AMCase Enzyme Control.

Measurement:

Measure fluorescence (Ex/Em = 320/445nm) in kinetic mode at 37 °C for 30 minutes. Choose two points (t_1 and t_2) in the linear range of the plot and obtain the corresponding fluorescence values (RFU_1 and RFU_2).

Calculation:

Calculate the slope for all samples, including Enzyme Control (EC), by dividing the net ΔRFU (RFU_2-RFU_1) values by the time Δt (t_2-t_1). Calculate % Relative Inhibitor and % Relative Activity as follows:

$$\% \text{ Relative Inhibition} = \frac{slope\ of\ (enzyme\ control\ [EC]) - slope\ of\ (test\ sample\ [S])}{slope\ of\ (enzyme\ control\ [EC])} \times 100$$

$$\% \text{ Relative activity} = \frac{slope\ of\ (test\ sample\ [S])}{slope\ of\ (enzyme\ control\ [EC])} \times 100$$

Technical Support

For all technical or commercial enquiries please go to:

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