Technical Bulletin

Lysozyme Inhibitor Screening Kit

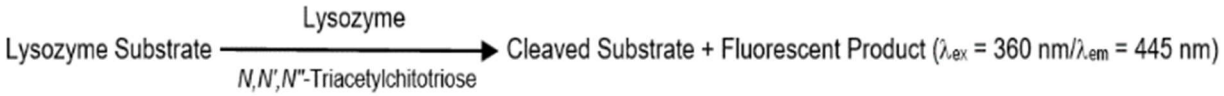
Catalog Number MAK393

Product Description

Lysozyme, also known as muramidase or N-acetylmuramide glycanhydrolase, is a glycoside hydrolase. It is ubiquitously found in a wide range of biological fluids such as tears, saliva, and serum, where it serves as a key effector of the innate immune system. It is also synthesized by certain carcinomas. The excessive production of lysozymes by cancer cells (especially myelomonocytic leukemia) results in higher levels of lysozyme which leads to renal failure. It has been demonstrated that the expression level of lysozyme is an independent prognostic factor which can be used to predict both relapse-free survival and overall survival in patients with breast carcinomas. Therefore, lysozyme inhibitors may constitute attractive, potential targets for developing anti-inflammatory and/or anti-tumor drugs.

The Lysozyme Inhibitor Screening Kit uses *N,N′,N′′*-Triacetylchitotriose, a competitive inhibitor that binds to the active site of lysozyme. The inhibitor decreases the catalytic ability of lysozyme to hydrolyze a fluorogenic substrate. The kit provides a simple, rapid, sensitive and reliable test suitable for screening of lysozyme inhibitors.

The kit is suitable for the screening, studying and characterizing of lysozyme inhibitors.



Components

The kit is sufficient for 100 fluorometric assays in 96 well plates.

- Lysozyme Assay Buffer 25 mL
  Catalog Number MAK393A
- Lysozyme Substrate (in DMSO) 65 µL
  Catalog Number MAK393B
- Lysozyme 1 vial
  Catalog Number MAK393C
- *N,N′,N′′*-Triacetylchitotriose 1 vial
  Catalog Number MAK393D

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (including multichannel pipettor)
- 96-well opaque flat-bottom plate. Cell culture or tissue culture treated plates are **not** recommended.
- Fluorescence multiwell plate reader

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C, protected from light. Briefly centrifuge small vials at low speed prior to opening.

Preparation Instructions

Lysozyme Assay Buffer: Warm to 37 °C before use. Store at either 2-8 ºC or -20 ºC.

Lysozyme Substrate (in DMSO): Aliquot and store at -20 ºC. Bring to room temperature prior to use.

Lysozyme (lyophilized): Reconstitute vial with 1060 µL of Lysozyme Assay Buffer and pipette up and down to mix thoroughly. Aliquot and store at -20 ºC. Avoid repeated freeze/thaw cycles. Keep on ice while in use. Stable for two months after reconstitution.

N,N′,N′′-Triacetylchitotriose: Reconstitute vial with 60 µL of purified water and store at -20 ºC. Use within two months of reconstitution.

Procedure

Note: To achieve better kinetic progress curves, pre-incubate the 96-well plate and assay buffer at 37 ºC prior to using.

Test Compound Preparation

1. Dissolve test inhibitors to 100× in appropriate solvent.
2. Further dilute 10-fold (10×) with Lysozyme Assay Buffer.
3. Add 10 µL of the diluted test inhibitor into designated wells (S).
4. Additional wells with serial dilutions of the test inhibitors may be prepared at this time, if desired, containing 10 µL in each candidate well if IC_{50} values need to be estimated.

Lysozyme Enzyme Control

Add 10 µL of purified water to a well designated as Enzyme Control (EC).

Inhibitor Control

Add 10 µL of *N,N′,N′′*-Triacetylchitotriose to a well designated as Inhibitor Control (IC).

Solvent Control

High solvent concentration might affect lysozyme activity. Prepare parallel well(s) as Solvent Control (SC) to test the effect of the solvent on lysozyme activity where water is substituted with the final solvent concentration in the samples.

Lysozyme Enzyme Solution Preparation

1. Mix enough reagents for the number of assays to be performed. For each well prepare 40 µL of Lysozyme Enzyme Solution according to Table 1. Mix well.

Table 1.
Preparation of Lysozyme Enzyme Solution

Reagent	Volume
Lysozyme Assay Buffer	30 µL
Lysozyme	10 µL

2. Add 40 µL of the Lysozyme Enzyme Solution into all wells, mix well.

Lysozyme Substrate Solution Preparation

1. Prepare an 80-fold dilution of Lysozyme Substrate by diluting 4 µL of Lysozyme Substrate with 316 µL of Lysozyme Assay Buffer. Vortex briefly and keep on ice.
2. Add 50 µL of the freshly diluted substrate to each well. Mix well. Always use freshly prepared substrate solutions. Do **not** store the diluted substrate solution.

Measurement

Measure fluorescence at λ_{Ex} = 360 nm/ λ_{Em} = 445 nm in kinetic mode at 37 ºC for 60 minutes. Choose two time points (T_1 and T_2) in the linear range of the plot and obtain the corresponding fluorescence values (RFU_1 and RFU_2).

Results

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).

For Solvent Controls that differ substantially from the EC, use their values in the equations below instead of EC. Calculate % Relative Inhibition as follows:

$$\% \text{ Relative Inhibition} = \frac{\text{Slope of [EC]} - \text{Slope of [S]}}{\text{Slope of [EC]}} \times 100\%$$

$$\% \text{ Relative Activity} = \frac{\text{Slope of [S]}}{\text{Slope of [EC]}} \times 100\%$$

Figure 1.
Progress curve of lysozyme activity in the presence or absence of the inhibitor *N,N′,N′′*-Triacetylchitotriose.

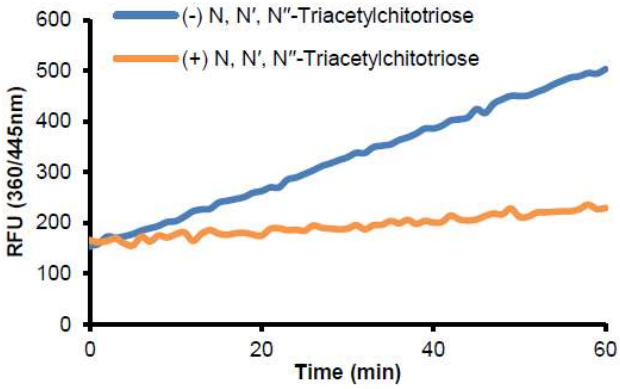
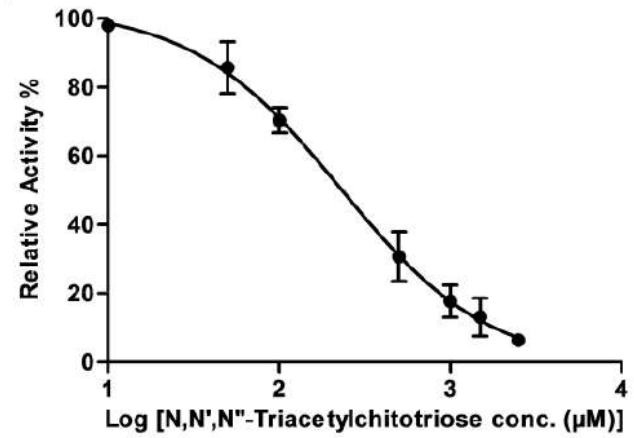


Figure 2.
IC_{50} of *N,N′,N′′*-Triacetylchitotriose was calculated to be 227.5 ± 1.2 μM. Assay was performed following the kit protocol.



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