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Lysozyme Inhibitor Screening Kit

rev 7/19

(Catalog # K237-100; 100 assays; Store at -20°C)

I. Introduction:

Lysozyme (EC3.2.1.17), 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, 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 causing 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. BioVision's 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.

$$\text{Lysozyme Substrate} \xrightarrow[\text{N,N′,N″-Triacetylchitotriose}]{\text{Lysozyme}} \text{Cleaved Substrate + Fluorescent Product (Ex/Em= 360/445 nm)}$$

II. Applications:

- Screening/studying/characterizing lysozyme inhibitors

III. Kit Contents:

Components	K237-100	Cap Code	Part Number
Lysozyme Assay Buffer	25 ml	WM	K237-100-1
Lysozyme substrate (in DMSO)	65 µl	Red	K237-100-2
Lysozyme (lyophilized)	1 vial	Green	K237-100-3
N,N′,N″-Triacetylchitotriose	1 vial	Brown	K237-100-4

IV. User Supplied Reagents and Equipment:

- 96-well white opaque plate with flat bottom
- 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 the entire protocol before performing the assay.

- **Lysozyme Assay Buffer:** Warm to 37°C before use. Store at either 4ºC or -20 ºC.
- **Lysozyme Substrate (in DMSO):** Aliquot and store at -20 ºC. Bring to room temperature before use.
- **Lysozyme (lyophilized):** Reconstitute with 1060 µl Lysozyme Assay Buffer, pipette up and down to mix thoroughly. Aliquot and store at -20°C. Avoid repeated freeze/thaw cycles. Keep on ice while in use. Stable for 2 months after reconstitution.
- ***N,N′,N″*-Triacetylchitotriose:** Reconstitute with 60 µl dH_2O and store at -20°C. Use within two months.

VI. Lysozyme Inhibitor Screening Assay Protocol:

1. **Screening Compounds, Inhibitor Control, and Enzyme Control Preparations:** Dissolve test inhibitors to 100X in proper solvent. Further dilute 10-fold (10X) in Lysozyme Assay Buffer. Add 10 µl diluted test inhibitor, *N,N′,N″*-Triacetylchitotriose or ddH_2O into wells assigned as test inhibitors (sample, S), and Inhibitor Control (IC). Add 10 µl ddH_2O to a well assigned as Lysozyme Enzyme Control (EC). 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.

	[S]	[EC]	[IC]
Test Inhibitor	10 µl	-	-
N, N′, N″-Triacetylchitotriose	-	-	10 µl
ddH_2O	-	10 µl	-

Notes:

a. 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.

b. To achieve better kinetic progress curves, we recommend preincubating the 96-well plate and assay buffer at 37ºC before using.

2. **Lysozyme Enzyme Solution Preparation:** For each well, prepare 40 µl Lysozyme Enzyme Solution.

Lysozyme Assay Buffer	30 µl
Lysozyme	10 µl

3. **Lysozyme Substrate Solution Preparation:** Prepare an 80-fold dilution of Lysozyme Substrate (i.e. Dilute 4 µl of Lysozyme Substrate with 316 µl of Lysozyme Assay Buffer), vortex briefly and keep on ice. Add 50 µl of the freshly diluted substrate to each well containing test sample, Inhibitor Control, Solvent Control and Lysozyme Enzyme Control. Mix well.

Note: Do not store the diluted substrate solution. Always use freshly prepared substrate solutions.

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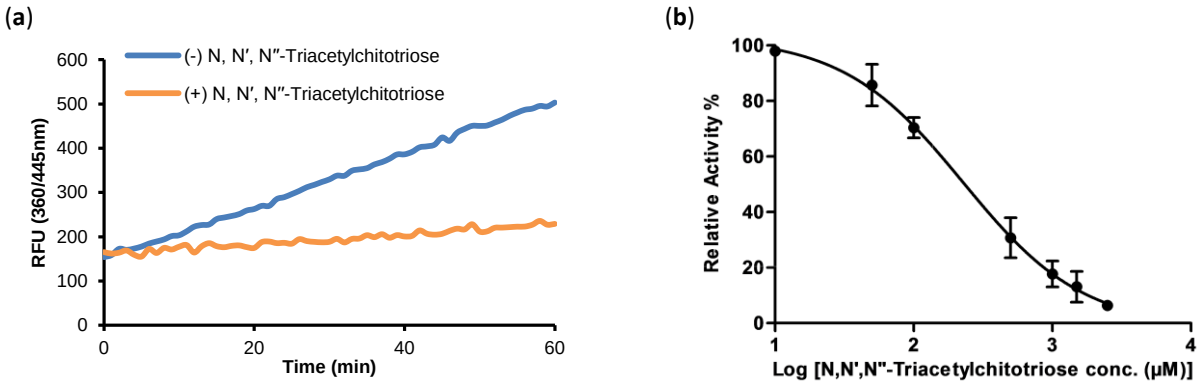
4. **Measurement:** Measure fluorescence (Ex/Em= 360/445nm) in kinetic mode at 37ºC for 60 min. Choose two time points (t_1 and t_2) in the linear range of the plot and obtain the corresponding fluorescence values (RFU_1 and RFU_2).
5. **Calculation:** Calculate the slope for all samples, including Enzyme Control (EC), by dividing the net ΔRFU (RFU_2-RFU_1) values by the time Δt (t_2-t_1). For Solvent Controls that differ substantially from the EC, use their values in the equations below instead of EC. Calculate % Relative Inhibition as follows:

$$\%\ \boldsymbol{Relative\ Inhibition} = \frac{\boldsymbol{Slope\ of\ [EC] - Slope\ of\ [S]}}{\boldsymbol{Slope\ of\ [EC]}} * \mathbf{100}$$

$$\%\ \boldsymbol{Relative\ Activity} = \frac{\boldsymbol{Slope\ of\ [S]}}{\boldsymbol{Slope\ of\ [EC]}} * \mathbf{100}$$



Figures: (a) Progress curve of lysozyme activity in the presence or absence of the inhibitor *N,N′,N″*-Triacetylchitotriose. (b) IC_{50} of *N,N′,N″*-Triacetylchitotriose was calculated to be 227.5 ± 1.2 µM. Assay was performed following the kit protocol.

VII. RELATED PRODUCTS:

Lysozyme Activity Assay Kit (K236)
EZLys™ Lysozyme, Human (8005)

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