ab303782 – Lysozyme Inhibitor Screening Kit

Screening/studying/characterizing lysozyme inhibitors.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab303782

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light.

Materials Supplied

Components	Quantity	Storage Condition
Lysozyme Assay Buffer	25 mL	-20°C
Lysozyme substrate (in DMSO)	65 µL	-20°C
Lysozyme (lyophilized)	1 vial	-20°C
N,N',N''-Triacetylchitotriose	1 vial	-20°C

Materials Required, Not Supplied

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

- 96-well white opaque plate with flat bottom).
- Multi-well spectrophotometer (fluorescence plate reader).

Reagent Preparation

Before using the kit, spin the tubes prior to opening. Read the entire protocol before performing the experiment.

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

Lysozyme Substrate (in DMSO): Aliquot and store at -20 °C. 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.

Assay Protocol:

Screening Compounds, Inhibitor Control, and Enzyme Control Preparations:

1. Dissolve test inhibitors (100-fold) in proper solvent.
2. Dilute 10-fold (10X) in Lysozyme Assay Buffer. Add 10 µl diluted test inhibitor, N,N',N''-Triacetylchitotriose or ddH2O into wells assigned as test inhibitors (sample, S), and Inhibitor Control (IC).
3. Add 10 µl ddH2O to a well assigned as Lysozyme Enzyme Control (EC).
4. Additional wells with serial dilutions of the test inhibitors may be prepared at this time if desired, containing 10 µl in each candidate per well if IC_{50} values needs to be estimated.

Δ Note:

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

Lysozyme Enzyme Solution Preparation:

For each well, prepare 40 µl Lysozyme Enzyme Solution, containing:

30 µl	Lysozyme Assay Buffer
10 µl	Lysozyme

Lysozyme Substrate Solution Preparation:

1. 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.
2. Add 50 µl of the freshly diluted substrate to each well containing: test sample, Inhibitor Control, Solvent Control and Lysozyme Enzyme Control. Mix well.

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

Measurement:

Measure fluorescence (Ex/Em= 360/445nm) in kinetic mode at 37°C for 60 min. Choose two time points (t1 and t2) in the linear range of the plot and obtain the corresponding fluorescence values (RFU1 and RFU2).

Calculation

Calculate the slope for all samples, including Enzyme Control (EC), by dividing the net ΔRFU (RFU2-RFU1) values by the time Δt (t2-t1). For Solvent Controls that differ substantially from the EC, use their values in the equations below instead of EC. Calculate % Relative Inhibition as follows:

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

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

For technical support contact information, visit: www.abcam.com/contactus