Beta-Lactamase Inhibitor Screening Microplate Assay Kit

Instructions for Use | Cat. No.: ARS6890

Screening/studying/characterizing beta-Lactamase Inhibitors for efficient treatment of antibiotic resistant bacterial infections.

For research use only . Not for diagnostic or therapeutic procedures .

1. Introduction

Beta-Lactamases (EC 3.5.2.6, βLs), are a large family of hydrolases comprising more than 850 identified members expressed in Gram-positive and Gram-negative bacteria.

βLs can be classified according to their substrate or inhibitor specificity. These enzymes are capable of hyd rolyzing four atom rings known as β- lactams. Antibiotics containing β -lactam rings (i.e. penicillin, cephalosporin, monobactam, carbapenem) are highly susceptible to be hydrolyzed via enzymatic activity, which deactivates their antibiotic potency. βLs have become a significant clinical threat due to the alarming number of cases of bacterial strains showing β- lactam antibiotic resistance. β-Lactamase inhibition has become an urgent target in the treatment of bacterial infections displaying β -Lactam resistance.Several β -Lactam derivatives have been reported as β- Lactamase inhibitors;however, only clavulanic acid, sulbactam and tazobactam have reached clinical importance.

Beta-Lactamase Inhibitor Screening Colorimetric Microplate Assay Kit provides a rapid, simple, sensitive, and reliable test suitable for high-throughput screening of β-Lactamase inhibitors. This kit is based on the hydrolysis of Nitrocefin, a chromogenic cephalosporin, in the presence of Tazobactam-a potent inhibitor of β-Lactamase, the rate of hydrolysis of the substrate is decreased.

2. Kit Components

Component	Volume	Storage
96-WellMicroplate	1 plate	-
Reaction Buffer	20 mlx 1	4 °C
Substrate	Powder x 1	-20 °C, keep in dark
Diluent	0.2 mL x 1	4 °C
Enzyme	Powder x 1	-20 °C, keep in dark
Inhibitor Control (Tazobactam)	Powder x 1	-20 °C
Technical Manual	1 Manual	-

3. Materials Required But Not Provided

1. Microplate reader to read absorbance at 490 nm
2. Distilled water
3. Pipettor , multi-channel pipettor
4. Pipette tips
5. Centrifuge
6. Timer

4. Reagent Preparation

Substrate: Warm Diluent to RT prior to use and centrifuge the Substrate tube briefly. Add 0.2 mL Diluent to dissolve before use and vortex, then add 0.8 mL Reaction Buffer mix. Store at -20°C and use within 1 month.

Inhibitor Control: Briefly centrifuge prior to opening. Add 1 mL Reaction Buffer to dissolve before use and vortex, the concentration will be 5 mmol/L. Store at -20°C. Use within 1 month.

Enzyme: Briefly centrifuge prior to opening.Add 1 mL Reaction Buffer to dissolve before use , then add 20 μL into 980 μL Reaction Buffer mix. Aliquot & store at -80°C and use within 1 month.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

5. Sample Preparation

Dissolve test inhibitors into proper solvent.

6. Assay Procedure

Warm all regents to room temperature before use . Add following reagents in to the microplate :

Reagent*	Sample**	Control***	Inhibitor Control	Blank
Reaction Buffer	60 μl	60 μl	60 μl	60 μl
Sample	20 μl	--	--	--
Distilled water	--	20 μl	--	30 μl
Inhibitor Control	--	--	20 μl	--
Enzyme	10 μl	10 μl	10 μl	--
Substrate	10 μl	10 μl	10 μl	10 μl
Mix, measure the absorbance in kinetic mode for 5-30 min. at 490 nm. Choose two time points (T1 & T2) in the linear range of the plot and obtain the corresponding values for the absorbance (Abs1 and Abs2).				

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

** Perform 5-fold serial dilutions of the samples as much as needed.

***The Control must be set up each time the assay is run.

7. Calculation

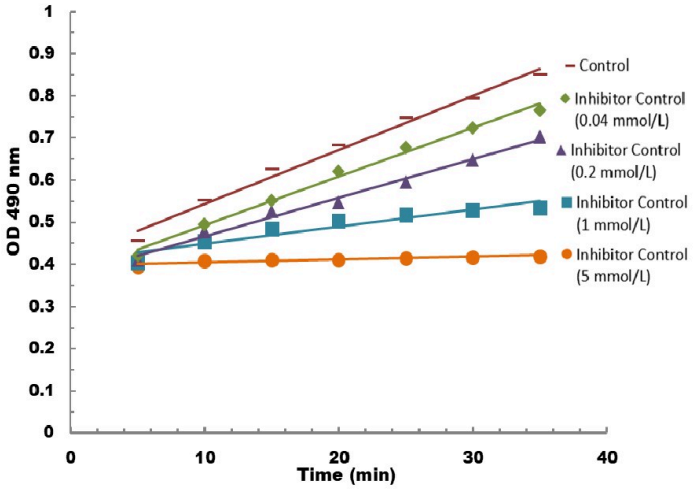
$$\text{Slope}_{\text{Control}} = \frac{\text{Abs2}_{\text{Control}} - \text{Abs1}_{\text{Control}}}{T_2 - T_1}$$

$$\text{Slope}_{\text{Sample}} = \frac{\text{Abs2}_{\text{Sample}} - \text{Abs1}_{\text{Sample}}}{T_2 - T_1}$$

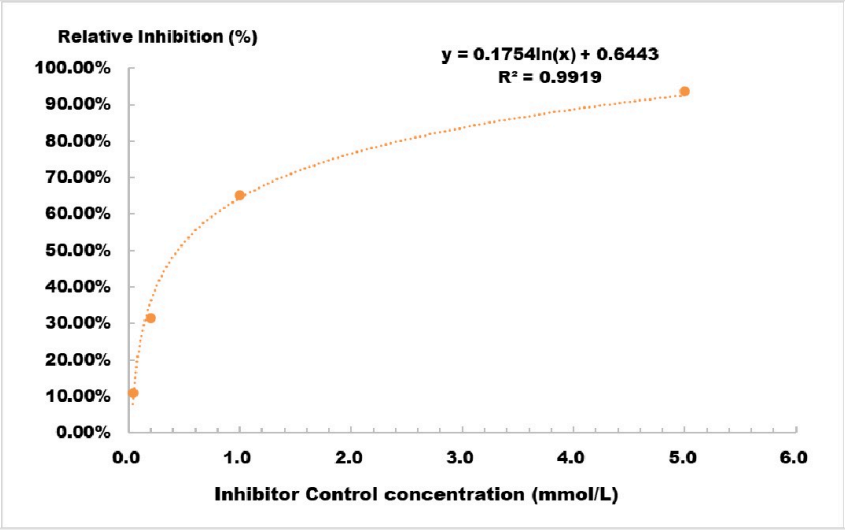
$$\text{Relative Inhibition (\%)} = \frac{(\text{Slope}_{\text{Control}} - \text{Slope}_{\text{Sample}})}{\text{Slope}_{\text{Control}}} \times 100\%$$

Item	Definition
$\text{Slope}_{\text{Sample}}$	the slope of the Sample Inhibitor
$\text{Slope}_{\text{Control}}$	the slope of the Enzyme Control
$\text{Abs1}_{\text{Sample}}$	the Sample Inhibitor absorbance at time point T1
$\text{Abs2}_{\text{Sample}}$	the Sample Inhibitor absorbance at time point T 2
$\text{Abs1}_{\text{Control}}$	the Enzyme Control absorbance at time point T1
$\text{Abs2}_{\text{Control}}$	the Enzyme Control absorbance at time point T 2

8. Typical Data



Inhibition of β-Lactamase Enzymatic Activity with Tazobactam



Different concentrations inhibitor control (Tazobactam) relative inhibition