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Beta-Lactamase Inhibitor Screening Kit (Colorimetric)

12/14

(Catalog # K804-100; 100 assays; Store at -20°C)

I. Introduction:

β-Lactamase (βL), (EC 3.5.2.6), is a bacterial enzyme that hydrolyzes the β-Lactam ring, a four-carbon ring, the building block of β-Lactam antibiotics. β-Lactams inhibit transpeptidases, enzymes that participate in the biosynthesis of bacterial cell walls. Penams, Carbapenems, Cephems, Clavams, Oxacephems and Monobactams have been widely used for the treatment of bacterial infections since the discovery of penicillin, the first β-Lactam antibiotic, and its bactericidal effect more than eighty years ago. However, an alarming number of cases of β-Lactam antibiotic-resistant infections have been reported. The resistance is found in bacterial strains producing β-Lactamase, which is responsible for diminishing the β-Lactam antibiotic potency. β-Lactamase inhibition, therefore, 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. BioVision's β-Lactamase Inhibitor Screening Kit utilizes the ability of the enzyme to hydrolyze Nitrocefin, a well-known β-Lactamase substrate. In the presence of Clavulanic Acid-a potent inhibitor of β-Lactamase, the rate of hydrolysis of the substrate is decreased. The kit provides a rapid, simple, sensitive, and reliable test suitable for high-throughput screening of β-Lactamase inhibitors.

II. Application:

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

III. Kit Contents:

Components	K804-100	Cap Code	Part Number
β-Lactamase Assay Buffer	25 ml	WM	K804-100-1
Nitrocefin (in DMSO)	0.1 ml	Red	K804-100-2
β-Lactamase (Lyophilized)	1 vial	Green	K804-100-3
Inhibitor Control (Clavulanic Acid, in DMSO)	50 µl	Blue	K804-100-4

IV. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Microplate reader

V. Storage and Handling:

Store kit at -20°C, protected from light. Avoid repeated freeze/thaw for all non-buffer components. Briefly centrifuge small vials prior to opening. Please read the entire protocol before using the kit.

VI. Reagent Preparation & Storage

- **Nitrocefin (in DMSO):** Ready to use. Protect from light. Keep at room temperature while in use.
- **β-Lactamase:** Dissolve the lyophilized β-Lactamase in 220 µl β-Lactamase Assay Buffer. Aliquot and store at -20°C. Avoid repeated freeze/thaw cycles. Use within two months. Keep on ice while in use.
- **Inhibitor Control (Clavulanic Acid):** Protect from light. Keep at room temperature while in use.

VII. β-Lactamase Inhibitor Screening Protocol:

1. **Screening Compounds, Inhibitor Control and Blank Control Preparations:** Dissolve test inhibitors into proper solvent. Dilute to 5X the desired test concentration with β-Lactamase Assay Buffer before use. Dilute Inhibitor Control 5-fold by adding 2 µl of Inhibitor Control into 8 µl of DMSO). To make working solution, further dilute 20X using β-Lactamase Assay Buffer (i.e. 1 µl of 5-fold diluted Inhibitor Control into 19 µl β-Lactamase Assay Buffer). Make as much as needed. Add 20 µl diluted test inhibitors, Inhibitor Control working solution, or β-Lactamase Assay Buffer into wells assigned as test inhibitors (Sample, S), Inhibitor Control (IC), or β-Lactamase Enzyme Control (EC) wells, respectively. Additional wells with serial dilutions of the test inhibitors may be prepared at this time if desired, containing 20 µl in each candidate well.

   Note: Preferred final solvent concentration should not be more than 2% by volume. If solvent exceeds 2% include a Solvent Control to test the effect of the solvent on enzyme activity.

2. **β-Lactamase Enzyme Solution Preparation:** For each well, prepare 50 µl β-Lactamase Enzyme Solution.

   48 µl β-Lactamase Assay Buffer
   2 µl β-Lactamase

   Mix. Add 50 µl/well into wells containing test inhibitors, Inhibitor Control & Enzyme Control. Incubate for 10 min. at 25ºC.

3. **Nitrocefin Substrate Solution Preparation:** For each well, prepare 30 µl of Nitrocefin substrate solution:

   29 µl β-Lactamase Assay Buffer
   1 µl Nitrocefin

   Mix and add 30 µl of the Nitrocefin Substrate Solution into each well. Mix well.

4. **Measurement**: Measure the absorbance in kinetic mode for 10-30 min. at 490 nm. Choose two time points (T_1 & T_2) in the linear range of the plot and obtain the corresponding values for the Absorbance (Abs_1 and Abs_2).

5. **Calculation:** Calculate the slope for all samples, including Enzyme Control (EC), by dividing the net ΔAbs (= Abs_2- Abs_1) values by the time ΔT (= T_2-T_1). Calculate % Relative Inhibition as follows:

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$$\% \textbf{ Relative Inhibition} = \frac{(\textbf{Slope of EC} - \textbf{Slope of S})}{\textbf{Slope of EC}} \textbf{ x 100}$$

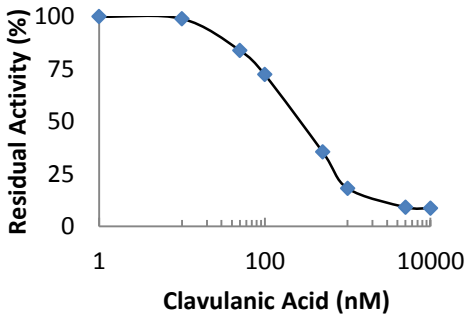

Figure: Inhibition of β-Lactamase Enzymatic Activity with Clavulanic Acid. Assay was performed following kit protocol.

VIII. RELATED PRODUCTS:

Beta-Lactamase Activity Colorimetric Assay Kit (K803) Nitrocefin (2388)
CENTA β-Lactamase substrate (2394)

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