ScreenQuest™ Beta-Lactamase Inhibitor Screening Kit

Catalog number: 11333
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 11333)
Component A: Nitrocefin	Freeze (< -15 °C), Minimize light exposure	1 Vial (100 uL)
Component B: β-Lactamase Assay buffer	Freeze (< -15 °C), Minimize light exposure	1 Bottle (10 mL)
Component C: β-Lactamase	Freeze (< -15 °C), Minimize light exposure	1 Vial (Lyophilized Powder)
Component D: Inhibitor, Avibactam	Freeze (< -15 °C), Minimize light exposure	1 Vial (1 mM, 50 uL/vial)

OVERVIEW

The ScreenQuest™ Beta-Lactamase Inhibitor Screening Kit is a precise and robust assay specifically designed for high-throughput screening (HTS) of β-lactamase inhibitors. The assay quantifies β-lactamase activity by measuring the hydrolysis of nitrocefin, a chromogenic cephalosporin substrate. Hydrolysis of nitrocefin by β-lactamase yields a colorimetric product with an absorbance maximum at 490 nm (A490), which is directly proportional to enzymatic activity. The assay is optimized for enzymatic studies and is fully compatible with automated HTS platforms, enabling reproducible, high-throughput workflows. This kit provides a reliable approach for identifying and characterizing β-lactamase inhibitors, supporting research into antimicrobial resistance mechanisms, and the development of novel therapeutic agents.

β-Lactamase originally described as penicillinase in *Escherichia coli*, has been identified in a wide range of bacterial species. These enzymes hydrolyze the β-lactam ring present in antibiotics such as penicillins, cephalosporins, monobactams, and carbapenems, effectively inactivating their antimicrobial properties. This enzymatic activity represents a major mechanism of bacterial resistance to β-lactam antibiotics, complicating the treatment of bacterial infections and emphasizing the need for innovative strategies to inhibit β-lactamase function.

AT A GLANCE

Protocol Summary

1. Prepare test samples and serially diluted β-Lactamase Inhibitor (Avibactam) standards (40 µL)
2. Add β-Lactamase working solution (10 µL)
3. Incubate at room temperature for 10 min
4. Add Nitrocefin working solution (50 µL)
5. Incubate at room temperature for 30-60 min
6. Monitor absorbance increase at OD ratio of 490/380 nm

Note: Bring kit components to room temperature before starting experiment.

KEY PARAMETERS

Absorbance microplate reader

Absorbance	490/380 nm
Recommended plate	Clear bottom

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

β-Lactamase stock solution (20X):

1. Add 50 µL of ddH2O to the vial of β-Lactamase (Component C) to prepare a 100 mU/mL β-Lactamase standard solution.

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/11333

Preparation of Standard Curve
1. Add 5 µL of β-Lactamase Inhibitor (Avibactam) (Component D) to 95 µL of β-Lactamase Assay Buffer (Component B) to prepare a 50 µM β-Lactamase Inhibitor Avibactam solution. 2. Add 12.5 µL of 50 µM β-Lactamase Inhibitor (Avibactam) to 487.5 µL of β-Lactamase Assay Buffer (Component B) to prepare a 1.25 µM β-Lactamase Inhibitor Avibactam solution (STD7). 3. Take 250 µL STD7 and perform 1:2 serial dilutions in β-Lactamase Assay Buffer (Component B) to create a series of Avibactam standards from STD7 to STD1 (1250 nM to 19.5nM).

PREPARATION OF WORKING SOLUTION

β-Lactamase Working Solution:

1. Add 10 µL of the β-Lactamase stock solution (20X) to 190 µL of the β-Lactamase Assay buffer to prepare β-Lactamase working solution.

   Note: 0.2 mL of β-Lactamase working solution can perform up to 20 tests in a 96-well plate. The β-Lactamase working solution is not stable and must be prepared fresh before each use to ensure reliability and accuracy.

Nitrocefin Working Solution:

1. Add 10 µL of Nitrocefin stock solution (Component A) to 1.0 mL of β-Lactamase Assay Buffer (Component B) and mix well to prepare Nitrocefin working solution.

   Note: 1mL Nitrocefin Working Solution can perform up to 20 tests in a 96-well plate.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of Avibactam standards and test samples in a 96- well solid black microplate.

Pos. Con.	Pos. Con.	TS	TS	....	....						
STD 7	STD 7	....	....	....	....						
STD 6	STD 6										
STD 5	STD 5										
STD 4	STD 4										
STD 3	STD 3										
STD 2	STD 2										
STD 1	STD 1	BL	BL								

Note: STD= Avibactam Standards, Pos. Con.=Positive Control (with β-Lactamase but no inhibitor), BL=Blank Control (Buffer only, No β-Lactamase), TS=Test Samples.

Table 2. Reagent composition for each well

Well	**Reagent:**	**β-Lactamase Working Solution**	**Description:**
STD7-STD1	Avibactam Serial Dilutions: 40 µL	10µL	Inhibitor: 1000 to 15.6 nM
Positive Control	β-Lactamase Assay Buffer: 40µL	10µL	Inhibitor: 0 nM (with β-Lactamase but no inhibitor)
TS	Test Sample: 40µL	10µL	Test Con. (nM)
Blank Control	β-Lactamase Assay Buffer: 50µL		0 nM (Substrate only, No β-Lactamase)

Note: Add the serially diluted Avibactam standards into the wells from STD 7 to STD 1 in duplicate.

Note: STD7-STD1 standard solutions range in concentration from 1250 nM to 19.5nM. After adding 10 µL β-Lactamase working solution, the final concentration of inhibitor in the 50µL mixture in each well will be from 1000 nM to 15.6 nM.

1. Add 40 µL Avibactam standards (STD7-1), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2.

   Note: For a 384-well plate, use 20 µL of reagent per well instead of 40 µL.

2. Add 10 µL β-Lactamase working solution to each well and incubate at room temperature for 10 min.

   Note: For a 384-well plate, use 5µL of β-Lactamase working solution per well.

3. Add 50 µL of Nitrocefin working solution to each well.

   Note: For a 384-well plate, add 25 µL Nitrocefin Working Solution to each well.

4. Incubate the reaction at room temperature for 30-60 minutes, protected from light.

5. Monitor the absorbance increase with an absorbance plate reader at OD ratio of 490/380 nm.

Data Analysis

The absorbance reading in the blank control wells (no β-Lactamase) serves as a baseline control, and the values are subtracted from the readings in other wells to account for background interference. The % inhibition is then calculated using the following formula:

$$\% \text{ Inhibition} = 100\% - \frac{(\text{Ratio of Sample} - \text{Ratio of Blank Control})}{(\text{Ratio of Positive Control} - \text{Ratio of Blank Control})} \times 100\%$$

EXAMPLE DATA ANALYSIS AND FIGURES

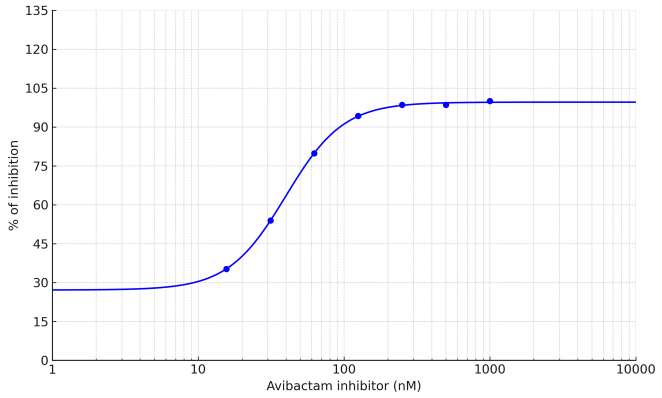

Figure 1. Avibactam dose response was measured with ScreenQuest™ Beta-Lactamase Inhibitor Screening Kit (Cat#11333) on a 96-well clear bottom plate using a SpectraMax reader (Molecular Devices).

DISCLAIMER

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