Amplite™ Colorimetric Beta-Lactamase Activity Assay Kit

Catalog number: 12551
Unit size: 200 Tests

Component	Storage	Amount
Component A: Nitrocefin	Freeze (< -15 °C), Minimize light exposure	1 vial (100 µL)
Component B: Assay Buffer	Freeze (< -15 °C)	1 bottle (10 mL)
Component C: β-Lactamase Standard	Freeze (< -15 °C), Minimize light exposure	1 vial (lyophilized powder)

OVERVIEW

β-Lactamases are a large family of enzymes capable of hydrolyzing β-lactams. β-Lactam ring is the common element in all beta-lactam antibiotics including penicillin derivatives, cephalosporins, monobactams, and carbapenems. Through hydrolysis, β-lactamase breaks the β-lactam ring open, thus deactivates the molecule's antibacterial properties. Bacteria from clinical and non-clinical settings are becoming increasingly resistant to β-lactam antibiotics by synthesizing β-lactamase. To overcome this resistance, β-lactam antibiotics are often given with β-lactamase inhibitors such as clavulanic acid. Therefore, detection of β-lactamase activity is of central importance to assess beta-lactam antibiotics as well as to prevent antibiotics resistance. AAT Bioquest's Colorimetric Beta-Lactamase Activity Assay Kit offers a sensitive colorimetric assay for measuring β-lactamase activity in biological samples. The β-lactamase activity is detected using Nitrocefin, which changes color from yellow to red upon hydrolysis by β-lactamase. The assay can be performed using an absorbance microplate reader by measuring the OD ratio at the wavelength of 490 nm to 380 nm.

AT A GLANCE

Protocol Summary

1. Prepare β-Lactamase standards or test samples (50 µL)
2. Add β-lactamase working solution (50 µL)
3. Incubate at RT for 30 - 60 min
4. Monitor absorbance increase at OD ratio of 490/380 nm

Important Thaw one vial of each kit component at room temperature before starting the 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 standard stock solution (50 mU/mL)
Add 100 µL of ddH_2O into the vial of β-Lactamase Standard (Component C) to make 50 mU/mL β-Lactamase standard solution.

PREPARATION OF STANDARD SOLUTION

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/12551

β-Lactamase standard
Add 10 µL of 50 mU/mL β-Lactamase standard solution into 990 µL 1x PBS buffer to generate 500 µU/mL β-Lactamase standard solution (SD7). Take 500 µU/mL β-Lactamase standard solution (SD7) and perform 1:2 serial dilutions in PBS to get serial diluted β-Lactamase standard (SD6 - SD1). Note: Diluted β-Lactamase standard solution is unstable, and should be used promptly.

PREPARATION OF WORKING SOLUTION

Add 50 µL Nitrocefin stock solution (Component A) into 5 mL of Assay Buffer (Component B) and mix well to make β-Lactamase working solution. **Note:** This β-Lactamase working solution is enough for one 96-well plate. The β-Lactamase working solution is not stable, prepare fresh for each use.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of β-Lactamase standards and test samples in a 96-well clear bottom microplate. SD= β-Lactamase Standards (SD1 - SD7, 7.8 to 500 µU/mL), BL=Blank Control, TS=Test Samples.

BL	BL	TS	TS
SD1	SD1	...	...
SD2	SD2	...	...
SD3	SD3		
SD4	SD4		
SD5	SD5		
SD6	SD6		
SD7	SD7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
SD1 - SD7	50 µL	Serial Dilutions (7.8 to 500 µU/mL)
BL	50 µL	1x PBS Buffer
TS	50 µL	test sample

1. Prepare β-Lactamase standards (SD), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.
2. Add 50 µL of β-Lactamase working solution to each well of β-Lactamase standard, blank control, and test samples to make the total β-Lactamase assay volume of 100 µL/well. For a 384-well plate, add 25 µL of β-Lactamase working solution into each well instead, for a total volume of 50 µL/well.
3. Incubate the reaction at room temperature for 30 - 60 minutes, protected from light.
4. Monitor the absorbance increase with an absorbance plate reader at OD ratio of 490/380 nm.

DISCLAIMER

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