Amplite™ Colorimetric Beta-Lactamase Activity Assay Kit

Ordering Information	*Storage Conditions*	*Instrument Platform*
Cat#: 12551 (200 Assays)	Keep in freezer and protect from light	Absorbance microplate readers

Introduction

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.

Kit Components

Components	**Amount**
Component A: Nitrocefin	1 vial (100 µL)
Component B: Assay Buffer	1 bottle (10 mL)
Component C: β-Lactamase Standard	1 vial (lyophilized powder)

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare β-lactamase assay mixture (50 µL) → Add β-lactamase standards or test samples (50 µL) → Incubate at RT for 30-60 min → Monitor absorbance increase at OD ratio of 490/380 nm

Note: Thaw one vial of each kit component at room temperature before starting the experiment.

1. Prepare β-lactamase standard stock solution:

Add 100 µL of ddH_2O into the vial of β-lactamase standard (**Component C**) to make 50 mU/mL β-lactamase standard stock solution.

Note: The unused β-lactamase *standard stock solution should be divided into single use aliquots and stored at -20 °C.*

2. Prepare serial dilutions of β-lactamase standard:

2.1 Add 10 µL of β-lactamase standard stock solution (50 mU/mL, from Step 1) into 990 µL 1×PBS buffer to generate 500 µU/mL β-lactamase standard solution.

Note: Diluted β-lactamase *standard solution is unstable, and should be used promptly.*

2.2 Take 200 µL of 500 µU/mL β-lactamase standard solution to perform 1:2 serial dilutions in PBS to get approximately 250, 125, 62.5, 31.3, 15.6, 7.8 and 0 µU/mL serial dilutions of β-lactamase standard.

2.3 Add serial dilutions of β-lactamase standard and β-lactamase containing test samples into a 96-well clear bottom microplate as described in Tables 1 and 2.

Table 1 Layout of β-lactamase standards and test samples in a 96-well clear bottom microplate

BL	BL	TS	TS	….	….						
Lac 1	Lac 1	….	….	….	….						
Lac 2	Lac 2										
Lac 3	Lac 3										
Lac 4	Lac 4										
Lac 5	Lac 5										
Lac 6	Lac 6										
Lac 7	Lac 7										

Note: Lac= β-Lactamase Standards, BL=Blank Control, TS=Test Samples.

Ordering: sales@aatbio.com; Tel: 800-990-8053 or 408-733-1055; Fax: 408-733-1304
Technical Support: support@aatbio.com; Tel: 408-733-1055

Table 2 Reagent composition for each well

β-Lactamase Standard	Blank Control	Test Sample
Serial Dilutions*: 50 µL	1×PBS Buffer : 50 µL	50 µL

**Note: Add the serially diluted β-lactamase standards from approximately 500 to 7.8 µU/mL into wells from Lac 1 to Lac 7 in duplicate.*

3. Prepare β-lactamase assay mixture:

Add 50 µL nitrocefin stock solution (**Component A**) into 5mL of **Component B**, and mix well to make β-lactamase assay mixture (**Component A+B).**

Note: This β-lactamase assay mixture is enough for one 96-well plate. The β-lactamase assay mixture is not stable, prepare fresh for each use.

4. Run β-lactamase assay:

4.1 Add 50 µL of β-lactamase assay mixture (from Step 3) to each well of β-lactamase standard, blank control, and test samples (see Step 2.3) to make the total volume of 100 µL/well.
Note: For a 384-well plate, add 25 µL of sample and 25 µL of β-lactamase assay mixture into each well.

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

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

Data Analysis

The absorbance reading in blank wells (with PBS and β-lactamase assay mixture only) is used as a control, and is subtracted from the values of those wells with the β-lactamase standards and test samples. A β-lactamase standard curve is shown in Figure 1. Calculate the β-lactamase concentrations of the samples according to the β-lactamase standard curve.

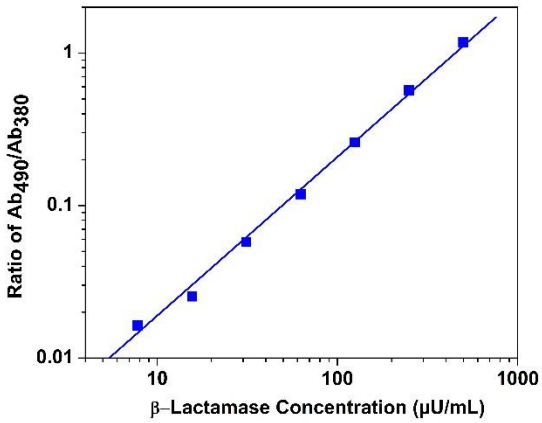

Figure 1. β-Lactamase dose response was measured with Amplite™ Colorimetric Beta-Lactamase Assay Kit (Cat#12551) on a 96-well clear bottom plate using a SpectraMax reader (Molecular Devices). As low as 15 µU/mL β-lactamase can be detected with 30 minutes incubation.

References

1. Wilke MS, Lovering AL, Strynadka NC. (2005) β-Lactam antibiotic resistance: a current structural perspective. Current Opinion in Microbiology 8(5): 525-533.
2. Drawz SM, Bonomo RA. (2010). Three Decades of β-Lactamase Inhibitors. Clinical Microbiology Reviews 23 (1): 160–201.
3. Fisher JF, Meroueh SO, Mobashery S. (2005). Bacterial Resistance to β-Lactam Antibiotics: Compelling Opportunism, Compelling Opportunity. Chemical Reviews 105 (2): 395–424.