Beta-Lactamase Activity Assay Kit (Colorimetric)

Catalog Number KA4556

100 assays

Version: 01

Intended for research use only
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Introduction

Background

Beta-Lactamases (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 hydrolyzing 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. Beta-Lactamase Activity Assay Kit offers a simple and sensitive assay that can detect and quantify the enzymatic activity of these hydrolases. The assay is based on the hydrolysis of Nitrocefin, a chromogenic cephalosporin, that results in the generation of a colored product (OD 490 nm), which is directly proportional to the amount of βL activity. The assay can detect enzymatic activity as low as 0.06 mU in a variety of biological samples.

![Beta-Lactamase Activity Assay Kit](Image)

Nitrocefin (Yellow) → Beta-Lactamase → Product (Red) (OD 490 nm)

✓ Application
- Measurement of β-Lactamase activity in various biological samples
- Analysis of β-Lactamase activity in pathological conditions

✓ Sample Type
- Serum, urine, saliva from mammals infected with βL-secreting bacteria
- Food (e.g. milk)
- Fermentation media, bacterial cultures, etc.
General Information

Materials Supplied

List of component

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>βL Assay Buffer</td>
<td>27 mL</td>
</tr>
<tr>
<td>Nitrocefin (in DMSO)</td>
<td>220 µL</td>
</tr>
<tr>
<td>Positive Control (Lyophilized)</td>
<td>1 vial</td>
</tr>
<tr>
<td>βL Hydrolysis Buffer</td>
<td>100 µL</td>
</tr>
</tbody>
</table>

Storage Instruction

Store the kit at -20°C, protected from light.

Materials Required but Not Supplied

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer
- DMSO

Precaution

For Research Use Only. Not to be used on humans.
Assay Protocol

Reagent Preparation

Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

✓ βL Assay Buffer and βL Hydrolysis Buffer: Warm βL Assay Buffer and βL Hydrolysis Buffer to room temperature before use.
✓ Nitrocefin (in DMSO): Warm to room temperature before use. Store at -20°C. Use within two months.

Sample Preparation

Liquid samples (i.e. biological fluids, fermentation media) can be assayed directly. Collect bacterial samples by centrifugation (10000 x g; 10 min) in a pre-weighed centrifuge tube. Remove supernatant and determine wet weight of the pellet. Resuspend the pellet in βL Assay Buffer using a minimum of 5 µL of βL Assay Buffer per mg of sample. Sonicate samples for 5 min. Keep samples on ice for 5 min. Remove insoluble material by centrifugation at 16000 x g at 4°C for 20 min. Collect the supernatant. Add 1-50 µL of supernatant into desired well(s) in 96-well plate. Adjust the volume to 50 µL/well with βL Assay Buffer. For Positive Control, dilute Positive Control 5-fold by adding 2 µL Positive Control to 8 µL of βL Assay Buffer. Add 1-10 µL of diluted Positive Control into desired well(s). Adjust the volume to 50 µL/well with βL Assay Buffer. Add 2-50 µL of undiluted serum to desired well(s) in a 96-well plate. Adjust the volume to 50 µL/well with 50 % DMSO (mix 500 µL 100% DMSO (provided) and 500 µL ddH₂O for about 20 wells).

Note: For unknown samples, we suggest doing a small pilot experiment & testing several doses to ensure the readings are within the Standard Curve linear range.

Assay Procedure

✓ Standard Curve Preparation

Hydrolyze Nitrocefin stock solution using βL Hydrolysis Buffer and DMSO (1:2:7) by adding 4 µL of Nitrocefin, 8 µL of βL Hydrolysis Buffer and 28 µL of DMSO (not provided) in an eppendorf tube. Incubate the reaction at 60°C for 10 min. Cool down the reaction to room temperature and briefly centrifuge the tube. Add 0, 2, 4, 6, 8 & 10 µL of the hydrolyzed Nitrocefin Standard (2 mM) into a series of wells in a 96-well plate to generate 0, 4, 8, 12, 16 & 20 nmol/well of Nitrocefin Standard. Adjust the volume to 100 µL/well with βL Assay Buffer.

Note: Prepare hydrolyzed Nitrocefin solution fresh every time. Discard unused hydrolyzed Nitrocefin.
Reaction Mix

Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µL Reaction Mix containing:

<table>
<thead>
<tr>
<th></th>
<th>Reaction Mix</th>
</tr>
</thead>
<tbody>
<tr>
<td>βL Assay Buffer</td>
<td>48 µL</td>
</tr>
<tr>
<td>Nitrocefin</td>
<td>2 µL</td>
</tr>
</tbody>
</table>

Mix well. Add 50 µL of the Reaction Mix to the wells containing samples and Positive Control(s).

Measurement

Measure the absorbance (OD 490 nm) kinetically at room temperature for 30-60 min, protected from light.

Note: Incubation time depends on the beta-Lactamase activity in samples. Longer incubation times may be required if sample’s βL activity is low.

We recommend measuring the OD in a kinetic mode, and choosing two time points (T₁ & T₂) in the linear range to calculate the beta-Lactamase activity of the samples. The Nitrocefin Standard Curve can be read in the Endpoint mode (i.e. at the end of the incubation time [60 min]).
Data Analysis

Calculation of Results

Subtract 0 Total Bilirubin Standard reading from all readings. Plot the Nitrocefin Standard Curve. Calculate the βL activity of the test sample: \( \Delta \text{OD} = A_2 - A_1 \) at a linear region of the curve. Apply the \( \Delta \text{OD} \) to the Nitrocefin Standard Curve to get \( B \) nmol of hydrolyzed Nitrocefin generated by βL during the reaction time (\( \Delta T = T_2 - T_1 \)).

**Sample βL Activity = \( \frac{B}{(\Delta T \times V) \times D} = \text{nmol/min/mL} = \text{mU/mL} \)**

Where:
- \( B \) is the amount of T Nitrocefin from the Standard Curve (nmol)
- \( \Delta T \) is the reaction time (min)
- \( V \) is the sample volume added into the reaction well (mL)
- \( D \) is the sample dilution factor

βL Activity can also be expressed as mU/mg of protein.

✓ Unit Definition

One unit of βL activity is the amount of enzyme that generates 1.0 µmol of Nitrocefin per min at pH 7.0 at 25°C.

![Graphs](image.png)

Figure: (a) Nitrocefin Standard Curve. (b) βL activity in E. coli culture (5 µL), contaminated media (CM; 30 µL) & Positive Control (4 µL). (c) βL Activity of E.Coli and contaminated media expressed per milligram of protein. Assay was performed following the kit protocol.