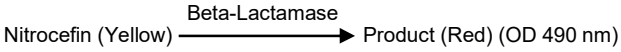
Beta-Lactamase Activity Colorimetric Assay Kit (#BN01019)

(Catalog # BN01019; 100 assays; Store at -20°C)

I. Introduction:

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. Assay Genie's 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.



II. Application:

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

III. Sample Type:

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

IV. Kit Contents:

Components	BN01019	Cap Code	Part Number
βL Assay Buffer	27 ml	WM	BN01019-1
Nitrocefin (in DMSO)	220 µl	Blue	BN01019-2
Positive Control (Lyophilized)	1 vial	Green	BN01019-3
βL Hydrolysis Buffer	100 µl	Purple	BN01019-4

V. User Supplied Reagents and Equipment:

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

VI. Storage and Handling:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

VII. Reagent Preparation and Storage Conditions:

- **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.
- **Positive Control:** Reconstitute with 20 µl βL Assay Buffer. Mix well. Aliquot & store at -20°C. Avoid repeated freeze/thaw. Stable for two months.

VIII. Beta-Lactamase Assay Protocol:

1. **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.

   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.

2. **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.

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

	Reaction Mix
βL Assay Buffer	48 µl
Ready-to-use Nitrocefin	2 µl

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

4. **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 kinetic mode, and choosing two time points (T_1 & T_2) in the linear range to calculate the beta-lactamase activity of the samples. The Nitrocefin Standard Curve can be read in Endpoint mode (i.e., at the end of the incubation time [60 min.]).

5. **Calculation:** Subtract 0 Standard reading from all Standard readings. Plot the Nitrocefin Standard Curve. Calculate the βL activity of the test sample: $\Delta OD = A_2 - A_1$ at a linear region of the curve. Apply the ΔOD 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 = B/(ΔT X V) x D = nmol/min/ml = mU/ml

Where: **B** is the amount of Nitrocefin from the Standard Curve (nmol)
ΔT 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.

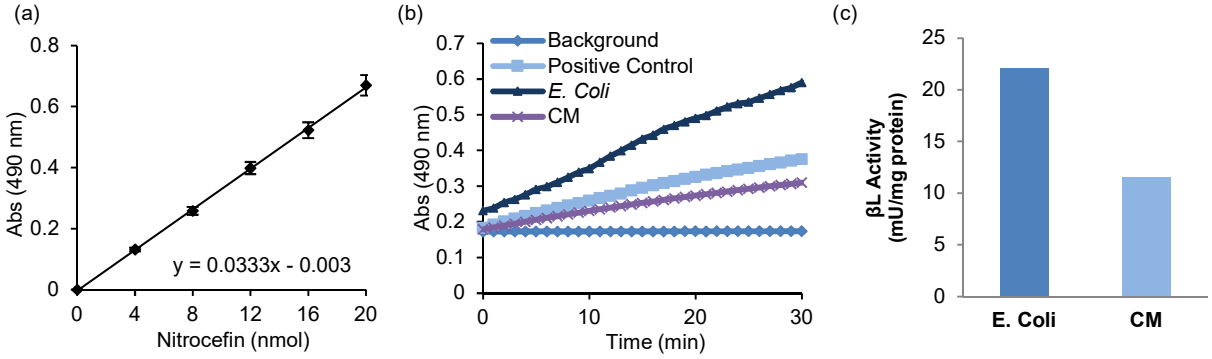


Figure: a) Nitrocefin Standard Curve. b) βL activity in *E. coli* culture (5 µl), contaminated media (CM; 30 µl) & Positive Control (4 µl). c) βL Activity of *E.Coli* and contaminated media expressed per milligram of protein. Assay was performed following the kit protocol.

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