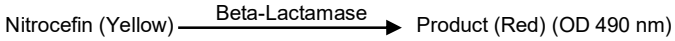
Beta-Lactamase Activity Colorimetric Assay Kit (384 well) (#BN01115)

(Catalog # BN01115; 400 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. Approaches for combatting this type of resistance have opened up the possibility of developing a new form of β lactam antibiotics that are more resistant to β-Lactamase enzymatic activity. Therefore, experimental research has been arising in wide extent for developing new drugs or inhibitors effective against the β-lactam antibiotic resistance bacteria. 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 15 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	BN01115	Cap Code	Part Number
βL Assay Buffer	27 ml	WM	BN01115-1
Nitrocefin (in DMSO)	220 µl	Blue	BN01115-2
Positive Control (Lyophilized)	1 vial	Green	BN01115-3
βL Hydrolysis Buffer	100 µl	Purple	BN01115-4

V. User Supplied Equipment and Reagents:

- 384-well clear plate with flat bottom
- Multi-well spectrophotometer with 384-well plate reading capability
- 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. Avoid repeated freeze/thaw.
- **Positive Control:** Dissolve in 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. Dissolve the pellet in βL Assay Buffer using a minimum of 50 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 1-10 µl of supernatant into desired well(s) in a 384-well plate. Adjust the volume to 15 µl/well with βL Assay Buffer. For Positive Control, dilute Positive Control 10-fold by adding 4 µl Positive Control to 36 µl of βL Assay Buffer. Add 2-10 µl of diluted Positive Control into desired well(s). Adjust the volume to 15 µl/well with βL Assay Buffer.

   Note:

   a. For unknown samples, we suggest doing a small pilot experiment & testing several doses to ensure the readings are within the Standard Curve linear range.
   b. Prepare parallel Nitrocefin background control well(s) (See step 3: Reaction Mix)

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 40 µl of βL Assay Buffer to make the stock of hydrolyzed Nitrocefin Standard 1 mM. Add 0, 1, 2, 3, 4 & 5 µl of the hydrolyzed Nitrocefin Standard (1 mM) into a series of wells in a 384-well plate to generate 0, 1, 2, 3, 4 & 5 nmol/well of hydrolyzed Nitrocefin Standard. Adjust the volume to 25 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. Dilute Nitrocefin stock 10 fold by βL Assay Buffer and use that as the new stock to prepare the reaction mix. For each well, prepare 10 µl Reaction Mix containing:

	Reaction Mix	Background Control Mix*
βL Assay Buffer	8.5 µl	23.5 µl
Diluted Nitrocefin	1.5 µl	1.5 µl

Mix well. Add 10 µl of the Reaction Mix to the wells containing samples and Positive Control(s).

*A Background Control Mix for the reaction mix is recommended to check if any significant background signal is generated from Nitrocefin.

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

Notes:

a. Incubation time depends on the Beta-Lactamase activity in samples. Longer incubation times may be required if sample's βL activity is low.

b. 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 [i.e. 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 Hydrolyzed Nitrocefin per min. at pH 7.0 at 25°C.

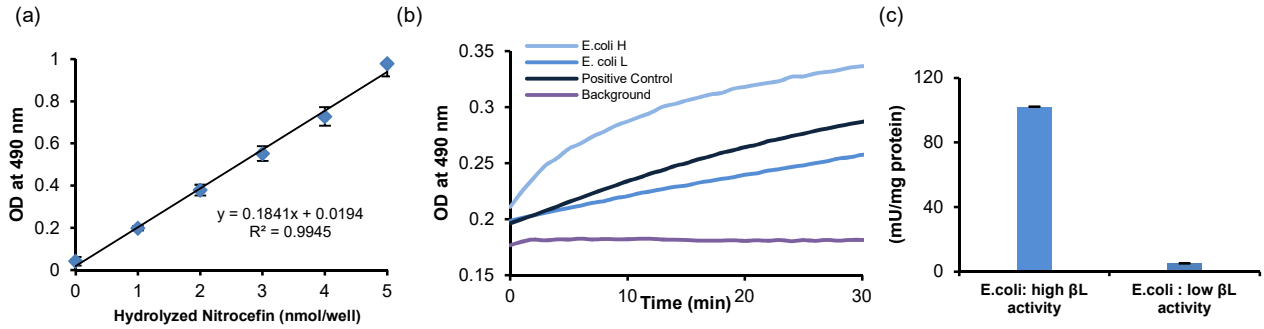


Figure: a) Nitrocefin Standard Curve. b) βL activity in *E. coli* H (*E. coli* strain with high βL activity) and *E.coli* L (*E. coli* strain with low βL activity). Samples were prepared following the kit's protocol then 1 µl for *E. coli* H and 10 µl of *E.coli* L were added to the well for the assay. Positive Control (2 µl) was added after diluting following the kit's protocol. Background control for Nitrocefin was prepared according to the kit's protocol. c) βL Activity of *E.coli* both higher and lower βL activity strains *of E.coli*.

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