

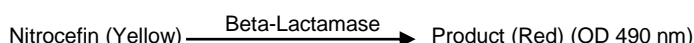
EZScreen™ Beta-Lactamase Activity Colorimetric Assay Kit (384 well)

(Catalog # K954-400; 400 assays; Store at -20°C)

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I. Introduction:

Beta-Lactamases (β Ls), are a large family of hydrolases comprising more than 850 identified members expressed in Gram-positive and Gram-negative bacteria. β Ls 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. β Ls 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. BioVision's EZScreen™ 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 Nitrocefim, a chromogenic cephalosporin, that results in the generation of a colored product (OD: 490 nm), which is directly proportional to the amount of β L 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 β L-secreting bacteria
- Food (e.g. milk)
- Fermentation media, bacterial cultures, etc.

IV. Kit Contents:

Components	K954-400	Cap Code	Part Number
β L Assay Buffer	27 ml	WM	K954-400-1
Nitrocefim (in DMSO)	220 μ l	Blue	K954-400-2
Positive Control (Lyophilized)	1 vial	Green	K954-400-3
β L Hydrolysis Buffer	100 μ l	Purple	K954-400-4

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:

- **β L Assay Buffer and β L Hydrolysis Buffer:** Warm β L Assay Buffer and β L Hydrolysis Buffer to room temperature before use.
- **Nitrocefim (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 μ l β L 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 β L Assay Buffer using a minimum of 50 μ l of β L 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 μ l of supernatant into desired well(s) in a 384-well plate. Adjust the volume to 15 μ l/well with β L Assay Buffer. For Positive Control, dilute Positive Control 10-fold by adding 4 μ l Positive Control to 36 μ l of β L Assay Buffer. Add 2-10 μ l of diluted Positive Control into desired well(s). Adjust the volume to 15 μ l/well with β L 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.
 - Prepare parallel Nitrocefim background control well(s) (See step 3: Reaction Mix)
- 2. Standard Curve Preparation:** Hydrolyze Nitrocefim stock solution using β L Hydrolysis Buffer and DMSO (1:2:7) by adding 4 μ l of Nitrocefim, 8 μ l of β L Hydrolysis Buffer and 28 μ l 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 μ l of β L Assay Buffer to make the stock of hydrolyzed Nitrocefim Standard 1 mM. Add 0, 1, 2, 3, 4 & 5 μ l of the hydrolyzed Nitrocefim Standard (1 mM) into a series of wells in a

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384-well plate to generate 0, 1, 2, 3, 4 & 5 nmol/well of hydrolyzed Nitrocefin Standard. Adjust the volume to 25 μ l/well with β L 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 β L Assay Buffer and use that as the new stock to prepare the reaction mix. For each well, prepare 10 μ l Reaction Mix containing:

	Reaction Mix	Background Control Mix*
β L Assay Buffer	8.5 μ l	23.5 μ l
Diluted Nitrocefin	1.5 μ l	1.5 μ l

Mix well. Add 10 μ l 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:

- Incubation time depends on the Beta-Lactamase activity in samples. Longer incubation times may be required if sample's β L 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 [i.e. 60 min.]

- 5. Calculation:** Subtract 0 Standard reading from all Standard readings. Plot the Nitrocefin Standard Curve. Calculate the β L 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 β L during the reaction time ($\Delta t = t_2 - t_1$).

$$\text{Sample } \beta\text{L Activity} = \frac{B}{(\Delta T \times V)} \times D = \text{nmol/min/ml} = \text{mU/ml}$$

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

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

Unit Definition: One unit of β L activity is the amount of enzyme that generates 1.0 μ mol of Hydrolyzed Nitrocefin per min. at pH 7.0 at 25°C.

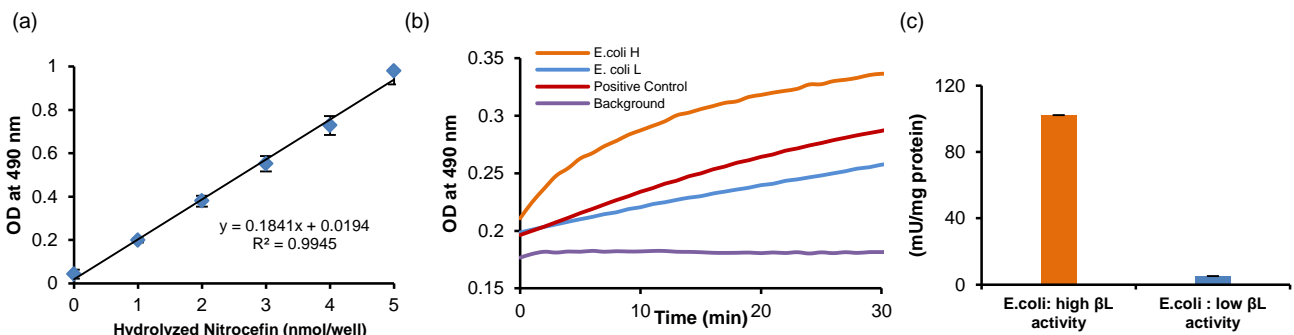


Figure: a) Nitrocefin Standard Curve. b) β L activity in *E. coli* H (*E. coli* strain with high β L activity) and *E. coli* L (*E. coli* strain with low β L activity). Samples were prepared following the kit's protocol then 1 μ l for *E. coli* H and 10 μ l of *E. coli* L were added to the well for the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

IX. Related Products:

- Avibactam Sodium (9569)
- CENTA β -Lactamase Substrate (2394)
- Nitrocefin (2388)
- Penicillin G Potassium, USP (2504)
- Penicillin G Sodium (2503)
- Beta-Lactamase Inhibitor Screening Kit (Colorimetric) (K804-100)
- Beta-Lactamase Activity Screening Kit (Colorimetric) (K803-100)

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