# Beta-Lactamase Activity

# Colorimetric MicroplateAssay Kit

# User Manual

## Catalog # CAK1319

(Version 1.2A)

Detection and Quantification of Beta-Lactamase(βLs) Activityin Tissue extracts, Cell lysate, Cell culture media and Other biological fluidsSamples.

**For research use only. Not for diagnostic or therapeutic procedures.**

## I. INTRODUCTION

Beta-Lactamases (EC 3.5.2.6, 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 Colorimetric Microplate Assay Kit provides a simple and direct procedure for measuring beta-Lactamase activity in a variety of samples. This kitisbased on the hydrolysis of Nitrocefin, a chromogenic cephalosporin, that results in the generation of a colored product, which is directly proportional to the amount of beta-Lactamase activity.

## II.KIT COMPONENTS

| Component | Volume | Storage |
|---|---|---|
| 96-WellMicroplate | 1 plate | |
| Assay Buffer | 30mlx 4 | 4 °C |
| Reaction Buffer | 20 mlx 1 | 4 °C |
| Substrate | Powder x 1 | -20 °C, keep in dark |
| Diluent | 0.5 ml x 1 | 4 °C |
| Hydrolysis Buffer | 5 ml x 1 | 4 °C |
| Standard | Powder x 1 | -20 °C, keep in dark |
| Positive Control | Powder x 1 | -20 °C |
| Technical Manual | 1 Manual | |

## III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 490 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

## IV. REAGENT PREPARATION

**Standard**:Briefly centrifuge prior to opening.Dissolvein0.1 ml Diluentto generate 10 mmol/L of standardstock solution.Keep in dark and store at 4 °C for 1-2 weeks or -20°C for 2-3months after reconstitution.Then dilute to 2 mmol/Lstandard top solutionby adding 20 µlstock solution into 80 µlReaction Buffer. Perform 2-fold serial dilutions of the top standardsolution using Reaction Bufferto make the standard curve. The concentration of standard curve could be 2/1/0.5/0.25/0.125/0.0625/0.0312mmol/L.

**Substrate**: Warm Diluent to RT prior to use,centrifuge the Substrate tube briefly,add 0.2 ml Diluentto dissolve before use and vortex, then add 0.8 ml Reaction Buffermix. Store at -20 °C. Use within 1 month.

**Positive Control**: Centrifuge the tube briefly, add 0.1 ml assay bufferto dissolve before use, then add 20 µl into 980 µl assay buffermix. Aliquot & store at -80 °C.Use within 1 month.

**Note:** Divide into small aliquots to avoid repeated freeze-thaw cycles.

## V. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay Buffer for 5 ×$10^6$ cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s,repeat 30 times); centrifuged at 8000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

Weighout 0.1 g tissue, homogenize with 1 mlAssay Buffer on ice, centrifuged at 8000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3.For liquid samples

Detect directly or dilute with Assay Buffer.

## VI. ASSAY PROCEDURE

Warm all regentsto room temperature before use.

Add following reagents intothe microplate:

| Reagent* | Sample** | Control | Standard | Blank | Positive Control |
|---|---|---|---|---|---|
| Standard | -- | -- | 20 µl | -- | -- |
| Distilled water | -- | -- | -- | 20 µl | -- |
| Hydrolysis Buffer | -- | -- | 80 µl | 80 µl | -- |
| Mix. | | | | | |
| Reaction Buffer | 170 µl | 170 µl | 100 µl | 100 µl | 170 µl |
| Sample | 20 µl | -- | -- | -- | -- |
| Assay Buffer | -- | 20 µl | -- | -- | -- |
| Positive Control | -- | -- | -- | -- | 20 µl |
| Substrate | 10 µl | 10 µl | -- | -- | 10 µl |
| Mix, keep in dark for 2 minutes at room temperature, record absorbance measured at 490 nm. | | | | | |

**Note:**

*Reagents must be added sequentially and should not be premixed prior to addition.

**For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

## VII. CALCULATION

**Unit Definition:**One unit of βLactivity is defined as the enzymegenerates1μmol of Nitrocefin per minuteat pH 7.0, 25 °C.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(OD_{Sample}-OD_{Control}) - \text{Intercept}}{\text{Slope}\times T} \times n(U/mL)$$

2. According to one point of the standard OD value and concentration

2.1 According to the protein concentration of sample

$$\text{Activity}=\frac{(C_{Standard}\times V_{Standard}) \times (OD_{Sample}-OD_{Control})}{(OD_{Standard}-OD_{Blank}) \times V_{Sample}\times C_{Protein}\times T}(U/mg)$$

2.2According to the quantity of cells or bacteria

$$\text{Activity}=\frac{(C_{Standard}\times V_{Standard}) \times (OD_{Sample}-OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times N\times(V_{Sample}/ V_{Assay})\times T}(U/10^4)$$

2.3 According to the weight of sample

$$\text{Activity}=\frac{(C_{Standard}\times V_{Standard}) \times (OD_{Sample}-OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times W \times(V_{Sample}/ V_{Assay})\times T}(U/g)$$

2.4 According to the volume of sample

$$\text{Activity}=\frac{(C_{Standard}\times V_{Standard}) \times (OD_{Sample}-OD_{Control})}{(OD_{Standard}-OD_{Blank}) \times V_{Sample}\times T}(U/mL)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Protein}$: the protein concentration of sample, mg/mL

W: the weight of total sample, g

N: the quantity of total cell or bacteria sample, $10^4$

$C_{Standard}$: the concentration of standard, μmol/mL

$V_{Standard}$: the volume of standard in assay procedure, mL

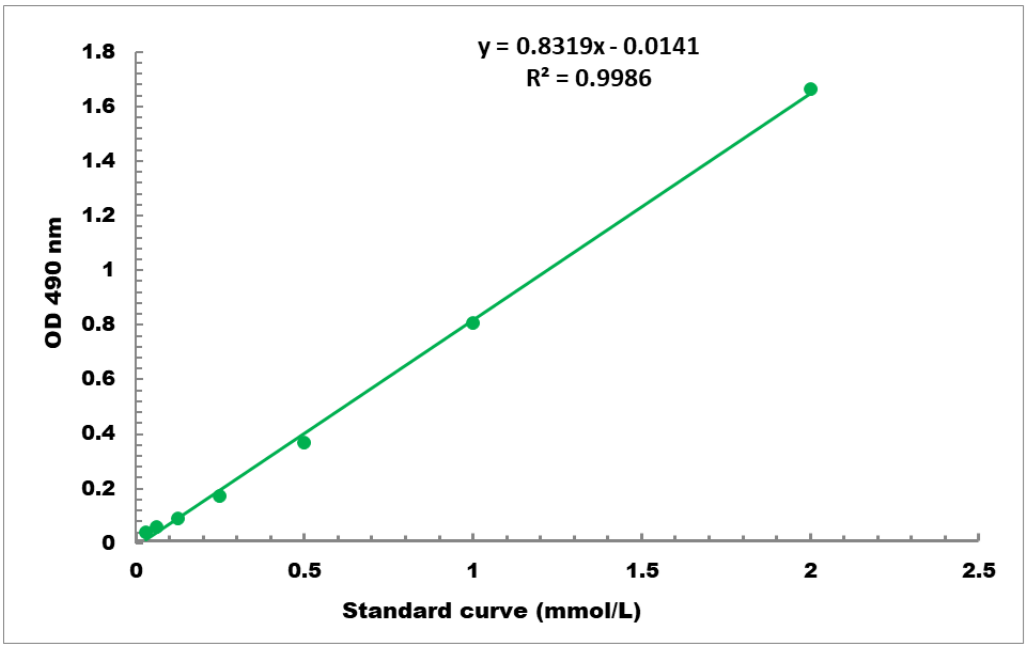
$V_{Sample}$: the volume of sample in assay procedure, mL

$V_{Assay}$: the volume of Assay Buffer in sample preparation, mL

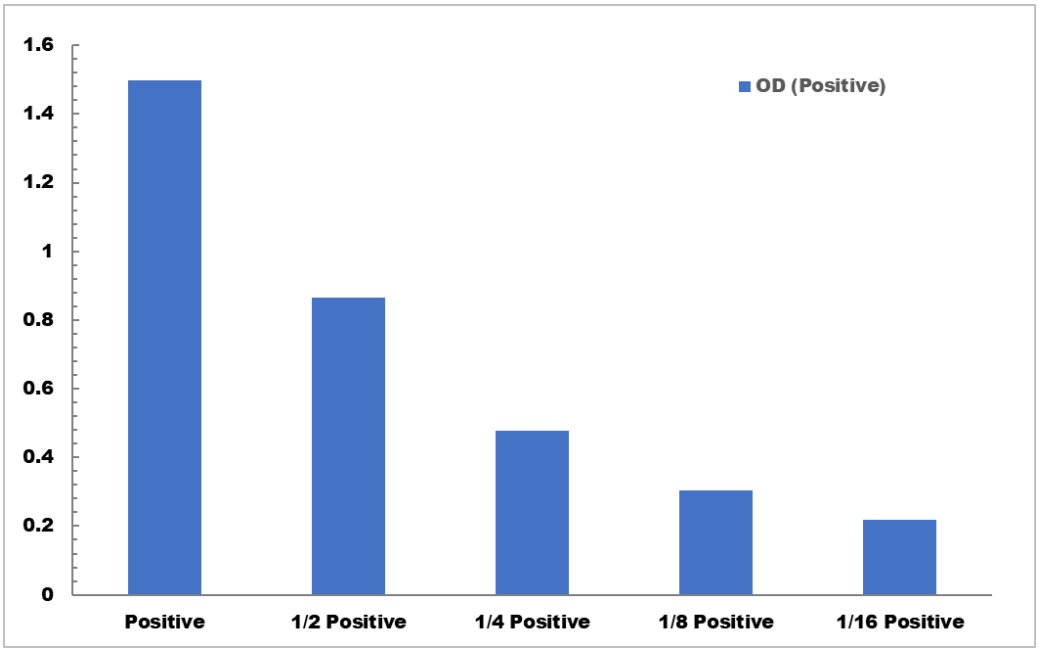
T: the reaction time, minute

## VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.




Detection Range: 0.02 mmol/L - 2 mmol/L




Positive Control reaction in 96-well plate assay with decreasing theconcentration