Beta-Lactamase Activity Microplate Assay Kit User Manual

Catalog # CAK1319

(Version 1.1A)

Detection and Quantification of Beta-Lactamase (βLs) Activity in Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

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

Cohesion BIOSCIENCES

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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 Microplate Assay Kit provides a simple and direct procedure for measuring beta-Lactamase activity in a variety of samples. This kit is based 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-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	20 ml x 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	

Note:

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

Standard: Centrifuge the tube briefly, add 0.1 ml Diluent to dissolve before use and vortex, then add 20 µl into 80 µl Reaction Buffer mix. The concentration will be 2 mmol/L. Dilute with Reaction Buffer to make the standard curve. Store at -20 °C. Use within one month.

Positive Control: add 0.1 ml assay buffer to dissolve before use, then add 20 µl into 980 µl assay buffer mix. Aliquot & store at -80 °C. Use within one month.

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. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay 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

Weigh out 0.1 g tissue, homogenize with 1 ml Assay 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.

V. ASSAY PROCEDURE

Warm all regents to room temperature before use.

Add following reagents into the 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:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) 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 sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

Unit Definition: One unit of βL activity is defined as the enzyme generates 1 μmol of Nitrocefin per minute at pH 7.0, 25 °C.

1. According to the protein concentration of sample

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

$= (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$

2. According to the weight of sample

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

$= (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$

3. According to the quantity of cells or bacteria

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

$= (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / N$

4. According to the volume of liquid

$\beta L\ (U/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$

$= (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$

$C_{Standard}$: the standard concentration, 2 mmol/L = 2 μmol/ml;

$V_{Standard}$: the volume of standard, 20 μl = 0.02 ml;

V_{Sample}: the volume of sample, 20 μl = 0.02 ml;

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

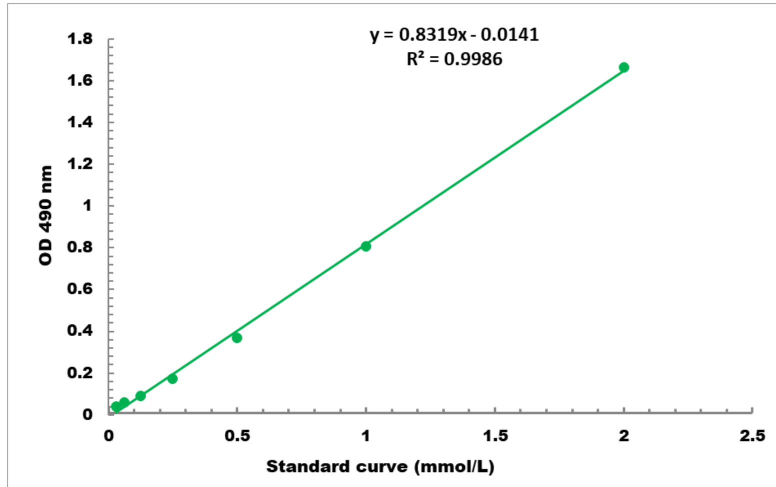
N: the quantity of cell or bacteria, $N \times 10^4$;

V_{Assay}: the volume of Assay buffer, 1 ml;

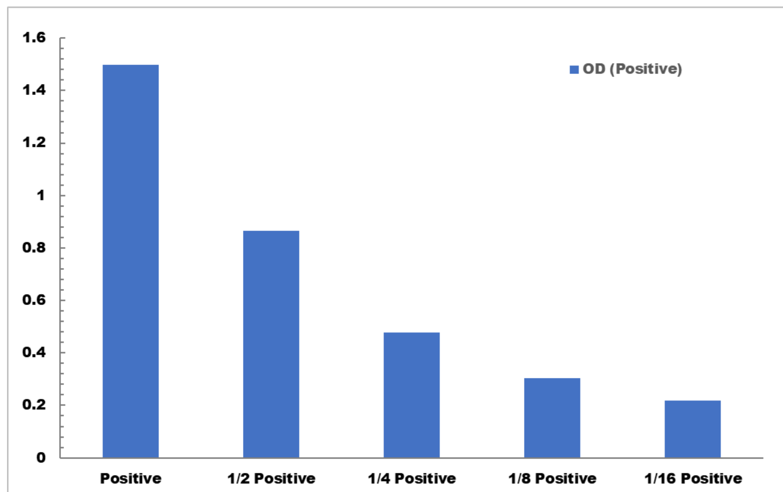
T: the reaction time, 2 minutes.

VII. 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 the concentration

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES