



## ProFoldin

10 Technology Drive, Suite 40, Number 188  
Hudson, MA 01749-2791 USA  
FAX: (508) 845-9258 Tel: (508) 735-2539  
www.profoldin.com  
[info@profoldin.com](mailto:info@profoldin.com)

# INSTRUCTIONS

## ProFoldin Beta Lactamase Assay Kit

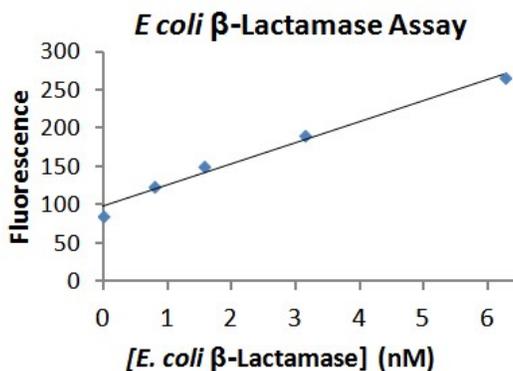
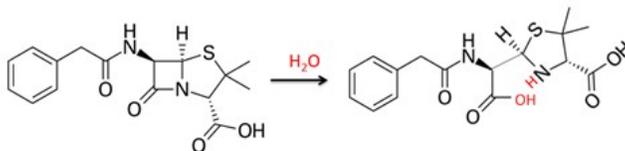
---

**Beta Lactamase Assay Kit**  
***E. coli* Beta Lactamase Assay Kit Plus**

**Catalog No. LAC100K**  
**Catalog No. LAC100KE**

### Introduction

Resistance to  $\beta$ -lactam antibiotics such as penicillins, cephalosporins, cephamycins and carbapenems are due to hydrolysis of the four-atom ring known as  $\beta$ -lactam by  $\beta$ -lactamases (penicillinase) produced by Gram-negative bacteria. Inhibition of  $\beta$ -lactamases prevents bacterial degradation of beta-lactam antibiotics. ProFoldin's high throughput assay for  $\beta$ -lactamases is based on binding of the dye with the hydrolyzed  $\beta$ -lactam that generates fluorescence at 535 nm (excitation at 485 nm). The assay can be performed in a 384-well or 96-well plate format for tests of  $\beta$ -lactamase activities and throughput screening of inhibitors against  $\beta$ -lactamases. The assay is not compatible with samples containing free amino acids or buffers containing DTT or EDTA.



The **Beta Lactamase Assay Kit (Catalog No. LAC100K)** includes 550  $\mu$ l of 10 x Buffer, 110  $\mu$ l of 50 x penicillin G and 260  $\mu$ l of 10 x fluorescence dye. The 10 x Buffer is composed of 100 mM HEPES, pH 7.5, 500 mM KCl. The kit is for 100 assays of  $\beta$ -lactamases in a 96-well plate or 384-well plate format. All assay reagents except the enzyme are included.

---



## ProFoldin

10 Technology Drive, Suite 40, Number 188

Hudson, MA 01749-2791 USA

FAX: (508) 845-9258 Tel: (508) 735-2539

[www.profoldin.com](http://www.profoldin.com)

[info@profoldin.com](mailto:info@profoldin.com)

# INSTRUCTIONS

---

The *E. coli* Beta Lactamase Assay Kit Plus (Catalog No. LAC100KE) includes 550  $\mu$ l of 10 x Buffer and 110  $\mu$ l of 50 x penicillin G, 260  $\mu$ l of 10 x fluorescence dye and 55  $\mu$ l of 100 x *E. coli*  $\beta$ -lactamases. It is for 100 assays of  $\beta$ -lactamases in a 96-well plate or 384-well plate format.

## ASSAY PROTOCOL

A 384-well plate (plate type Matrix 4318 or alike) or 96-well plate (plate type: Costar 3915 or alike) may be used for the fluorescence-based assay. The reaction volume is 50  $\mu$ l and the final assay volume is 75  $\mu$ l.

- Reaction:** Prepare each reaction solution by mixing 43.5  $\mu$ l of H<sub>2</sub>O, 5  $\mu$ l of 10 x Buffer, 1  $\mu$ l of 50 x penicillin G and 0.5  $\mu$ l of 100 x  $\beta$ -lactamase. Incubate the reaction mixture at 37°C for 60 min.
- Detection:** Dilute the 10 x fluorescence dye with water to make the 1 x dye. Mix 25  $\mu$ l of the 1 x dye with 50  $\mu$ l of the reaction mixture at the end of the reaction. Measure the fluorescence intensity at 535 nm using the excitation wavelength at 485 nm in 5 min.

## Assay protocol for enzyme inhibition

The assay can be optimized in terms of assay window, assay linearity and sensitivity to competitive inhibitors. ProFoldin offers HTS assay development service. For more information, please visit our website at <http://www.profoldin.com/services.html>.

## Related products

PST100	Penicillin Drug Stability Test Kit
UPA1000	Ultra-sensitive High-throughput Protease Assay Kit
TBP100KE	M. Tuberculosis ClpP Protease Assay Kit Plus
HMP100KE	Human Mitochondrial ClpP Protease Assay Kit Plus
HDPA100KE	Human DNA Polymerase Alpha Assay Kit Plus
DPB100KE	Human DNA Polymerase Beta Assay Kit Plus
DPG100KE	Human DNA Polymerase Gamma Assay Kit Plus
RPA100KE	<i>E. coli</i> RNA Polymerase Assay Kit Plus
RPA100KSE	<i>S. aureus</i> RNA Polymerase Assay Kit Plus
MRPA100K	Human Mitochondrial RNA Polymerase Assay Kit
MRPA100KE	Human Mitochondrial RNA Polymerase Assay Kit Plus
T7RPA100KE	T7 RNA Polymerase Assay Kit Plus
AMV100KE	AMV Reverse Transcriptase Assay Plus
MLV100KE	M-MLV Reverse Transcriptase Assay Plus
HIV100KE	HIV Reverse Transcriptase Assay Plus
VRT100K	Viral RNA-dependent RNA Polymerase Assay Kit – 100

For more information of drug targets and enzyme assays, please visit [www.profoldin.com](http://www.profoldin.com) or contact us at [info@profoldin.com](mailto:info@profoldin.com).