Technical Bulletin

Beta-Lactamase Activity Assay Kit

Catalogue Number MAK545

Product Description

β-Lactamases are a large family of enzymes capable of hydrolyzing β-lactams. A β-Lactam ring is the common element in all beta-lactam antibiotics including penicillin derivatives, cephalosporins, monobactams, and carbapenems. Through hydrolysis, β-lactamase breaks the β-lactam ring open, deactivating the antibacterial properties. Bacteria from clinical and non-clinical settings are becoming increasingly resistant to β-lactam antibiotics by synthesizing β-lactamase. To overcome this resistance, β-lactam antibiotics are often given with β-lactamase inhibitors such as clavulanic acid. Therefore, detection of β-lactamase activity is of central importance to assess beta-lactam antibiotics as well as to prevent antibiotics resistance.

The β-Lactamase Activity Assay Kit offers a sensitive colorimetric assay for measuring β-lactamase activity in biological samples. The β-lactamase activity is detected using nitrocefin, which changes color from yellow to red upon hydrolysis by β-lactamase. The assay can be performed using an absorbance microplate reader by measuring the OD ratio at the wavelength of 490 nm to 380 nm.

Components

The kit is sufficient for 200 colorimetric assays in 96-well plates.

- Nitrocefin 100 µL
  Catalogue Number MAK545A
- Assay Buffer 10 mL
  Catalogue Number MAK545B
- β-Lactamase Standard 1 Vial
  Catalogue Number MAK545C

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories.
- Spectrophotometric multiwell plate reader.
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes
- Phosphate Buffered Saline
  (Catalogue Number PPB006 or equivalent)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped in wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate to room temperature prior use.

Procedure

All Samples and Standards should be run in duplicate.

Reagent Preparation

Note: Unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

Preparation of Stock Solution

β-Lactamase Standard Stock Solution (50 mU/mL): Add 100 µL of purified H_2O into the vial of β-Lactamase Standard to make 50 mU/mL β-Lactamase Standard Solution.

Preparation of β-Lactamase Standard Solution

1. Add 10 µL of the 50 mU/mL β-Lactamase Standard solution into 990 µL 1x PBS buffer to generate 500 µU/mL β-Lactamase Standard solution (SD1).
2. Take 500 µU/mL β-Lactamase Standard solution (SD1) and perform 1:2 serial dilutions in PBS to get serial diluted β-Lactamase Standard (SD2 - SD7) as shown in Table 1.

Note: Diluted β-Lactamase Standard solution is unstable and should be used immediately.

Table 1.
Serial Dilution of β-Lactamase Standard (SD)

Dilution	Std Volume (µL)	Serial dilution Source	1x PBS Volume (µL)	Conc (µU/ml)
SD1	300	500 µU/mL stock	0	500
SD2	150	From SD1	150	250
SD3	150	From SD2	150	125
SD4	150	From SD3	150	62.5
SD5	150	From SD4	150	31.25
SD6	150	From SD5	150	15.62
SD7	150	From SD6	150	7.81

Preparation of Working Solution

β-Lactamase Working Solution: Add 50 µL of Nitrocefin into 5 mL of Assay Buffer and mix well.

Note: This β-Lactamase working solution is enough for one 96-well plate. The β-Lactamase working solution is not stable, prepare fresh for each use.

Assay Reaction

1. Add 50 µL of each β-Lactamase Standard, test Sample, and blank into separate wells of a 96-well clear bottom plate. Use PBS for the blanks.
2. Add 50 µL of β-Lactamase Working solution to each well of β-Lactamase Standard, blank, and test Samples to make the total assay volume of 100 µL/well.

Measurement

1. Incubate the reaction mixture at room temperature for 30 - 60 minutes protected from light.
2. Monitor the absorbance increase with an absorbance plate reader with path check on at OD of 490 nm to 380 nm.

Results

1. Subtract the blank value from the Standard and Sample readings to obtain the baseline corrected values.
2. Use the values obtained from the Standards to plot a Standard curve.
3. The amount of β-Lactamase activity present in the Samples may be determined from the Standard curve.

Unit Definition: One unit of β-lactamase is the amount of enzyme required to hydrolyze 1.0 µmole of nitrocefin per minute at pH 7.0 at 25 °C.

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