Technical Bulletin

β-Lactamase Inhibitor Screening Kit

Catalogue Number MAK222

Product Description

β-Lactamase (beta-lactamase, βL, EC 3.5.2.6) is an enzyme first identified in *Escherichia coli* and has been described as penicillinase. A number of βLs have since been identified from various bacteria. β-Lactamases specifically hydrolyze β-lactam rings present in antibiotics such as penicillin, cephalosporins, monobactam, and carbapenem, and confer resistance against these antibiotics.[1,2]

The β-Lactamase Inhibitor Screening Kit is a rapid, simple and sensitive assay that is suitable for high throughput screening of β-Lactamase inhibitors. βL activity is measured by hydrolyzing a chromogenic cephalosporin called nitrocefin, producing a colorimetric product (A490), proportional to the enzymatic activity present.

Components

The kit is sufficient for 100 assays in 96 well plates.

β-Lactamase Assay Buffer Catalog Number MAK222A	25 mL
Nitrocefin, in DMSO Catalog Number MAK222B	0.1 mL
β-Lactamase Catalog Number MAK222C	1 vl
Inhibitor Control, Clavulanic acid Catalog Number MAK222D	1 vl

Reagents and Equipment Required but Not Provided

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader
- Dimethyl sulfoxide (DMSO)

Precautions and Disclaimer

This product is 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.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

β-Lactamase Assay Buffer – Allow buffer to come to room temperature before use.

Nitrocefin (in DMSO) – Store at -20 °C. Protect from light. Warm to room temperature before use to melt DMSO. Use within 2 months.

β-Lactamase – Reconstitute in 220 µL of β-Lactamase Assay Buffer. Aliquot and store at -20 °C. Keep on ice during use. Use within 2 months.

Inhibitor Control – Prepare Inhibitor Control Stock Solution by diluting Inhibitor Control 5X in DMSO. Aliquot and store at -20 °C.

Storage/Stability

The kit is shipped on wet ice. Storage at -20 °C, protected from light, is recommended.

Procedure

Sample Preparation

Prepare a 5X Sample Inhibitor Solution by mixing Sample inhibitors with β-Lactamase Assay Buffer to 5X the final testing concentration. An initial concentrated inhibitor solution may be in a different solvent if the inhibitor is minimally soluble in the aqueous β-Lactamase Assay Buffer.

For unknown inhibitor Samples, it is suggested to test several inhibitor concentrations.

An Inhibitor Control may be prepared by diluting Inhibitor Control Stock Solution 20X in β-Lactamase Assay Buffer.

If solvent content is greater than 2%, include a Solvent Control by preparing a solution with the same dilutions as the Sample but omitting the Sample inhibitor.

To correct for background in Samples, include a Sample Blank by omitting the β-Lactamase. The Sample Blank readings can then be subtracted from the Sample readings.

Prepare an Enzyme Control (uninhibited enzyme) by using β-Lactamase Assay Buffer in place of Sample inhibitor.

Add 20 µL of Sample inhibitor (5X Sample Inhibitor Solution), Sample Blank (5X Sample Inhibitor Solution), Enzyme Control (β-Lactamase Assay Buffer), or Inhibitor Control into duplicate wells of a 96 well plate.

Assay Reaction

1. Set up Inhibition Reaction Mixes according to the scheme in Table 1. 50 µL of the appropriate Inhibition Reaction Mix is required for each reaction (well).

Table 1.

Inhibition Reaction Mixes

Reagent	Samples and Controls	Sample Blank
β-Lactamase Assay Buffer	48 µL	50 µL
β-Lactamase	2 µL	–

2. Add 50 µL of the appropriate Inhibition Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting. Incubate the plate at 25 °C for 10 minutes. Protect the plate from light during the incubation.
3. Set up an Enzymatic Reaction Mix according to the scheme in Table 2. 30 µL of the Enzymatic Reaction Mix is required for each reaction (well).

Table 2.

Enzymatic Reaction Mix

Reagent	Samples, Controls, and Sample Blank
β-Lactamase Assay Buffer	29 µL
Nitrocefin	1 µL

4. Add 30 µL of the Enzymatic Reaction Mix to each reaction well. Mix well using a horizontal shaker or by pipetting.
5. Measure the absorbance (ABS, A_{490}) in a microplate reader in kinetic mode for 10–30 minutes. It is recommended to take absorbance readings every minute.

Results

Calculations

Plot the absorbance (ABS) for each well versus time.

Choose two time points (T1 and T2) in the linear range of the plot and obtain the slope for each well between T1 and T2. Determine the ABS at each time (ABS1 and ABS2) and use them to determine the slope of the plot (ABS/minute).

Note: The Enzymatic Control must be set up each time the assay is run.

Subtract the slope of the Sample Blank from the slope of the samples to obtain the corrected measurement. Use the corrected measurement to determine the % Relative Inhibition.

% Relative Inhibition

Slope = (ABS2 – ABS1)/(T2 – T1) = ΔABS/minute

$$\% \text{ Relative Inhibition} = \frac{(\text{Slope}_{EC} - \text{Slope}^{SM})}{\text{Slope}_{EC}} \times 100\%$$

where:

Slope_{SM} = the slope of the Sample Inhibitor

Slope_{EC} = the slope of the Enzyme Control

Note: Irreversible inhibitors that completely inhibit β-Lactamase activity will have ΔABS = 0.
The % Relative Inhibition will be 100%.

Sample Calculation

SlopeSM = 0.435 ABS/min

SlopeEC = 0.755 ABS/min

$$\% \text{ Relative Inhibition} = \frac{(0.755 - 0.435)}{0.755} \times 100\%$$

$$= 42.4\%$$

References

1. Abraham, E.P., *et al*., An enzyme from bacteria able to destroy penicillin. Nature, **146**, 837 (1940).
2. Drawz, S.M., *et al*., Three decades of beta-lactamase inhibitors. Clin. Microbiol. Rev., **23**, 160–201 (2010).

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For Colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mixes before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the Reaction Mixes
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare Reaction Mixes whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

Notice

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