

EZER™ CTX-M β -lactamases Rapid Test

INTENDED USE

The **EZER™** CTX-M β -lactamases Rapid Test is a qualitative rapid immunoassay for the detection of CTX-M groups 1, 2, 8, 9 and 25 in a bacterial colony obtained from culture. It is an *in vitro* diagnostic assay, for professional use only, that aids to detect extended-spectrum β -lactamases (ESBLs) produced by Enterobacterales.

BACKGROUND

The production of extended-spectrum β -lactamases (ESBLs) is the main mechanism of bacterial resistance to β -lactamide antibiotics. In recent years, with the widespread use of cephalosporins, the spread of CTX-M ESBLs has become strong, causing popularity and spread in many countries and regions. It has gradually become the main type of ESBLs epidemic in many countries, and poses a great threat to human and animal health¹.

Currently, more than 170 CTX-M variants have been identified and described in 5 main groups (CTX-M groups 1, 2, 8, 9 and 25). Although the dominant variants of CTX-Ms are geographically different, CTX-M-15 and CTX-M-14 are the most common variants detected worldwide in clinically important pathogens, followed by CTX-M-2, CTX-M-3 and CTX-M-1².

CTX-M β -lactamases Rapid Test is an *in vitro* rapid and visual multiplex immunochromatographic assay that detects CTX-M β -lactamases in bacterial colonies, ensures a more rational use of last resort antibiotics such as carbapenems.

PRINCIPLE

The test is ready to use and is based on a membrane technology with colloidal gold nanoparticles. Our kit is aimed to the detection of CTX-M groups 1, 2, 8, 9 and 25 β -lactamases in bacterial culture.

A nitrocellulose membrane is sensitized with a monoclonal antibody directed against CTX-M β -lactamases ("T" line) and control capture reagent ("C" line).

Monoclonal antibodies that individually recognize CTX-M β -lactamases label with colloidal gold and dried on a membrane.

When the provided buffer containing the resuspended bacteria comes into contact with the strip, the solubilized antibody-colloidal gold conjugates migrate with the sample by passive diffusion, while conjugates and

sample material come into contact with the immobilized respective antibodies that are adsorbed onto the nitrocellulose strip. If the sample contains an CTX-M β -lactamases, the complexes made of the conjugates and CTX-M β -lactamases will remain bound to their "T" lines. Solution continues to migrate to reach a second reagent (control reagent) that binds the migration excess conjugates, thereby producing a red control line that confirms that the test is valid. The result is visible within 15 minutes in the form of red lines on the strip.

CONTENTS

Test Device (20 Pcs)
Extraction Tube with Buffer (20 Pcs)
Package Insert (1 Pc)

Warnings and Precautions

1. For *in vitro* Diagnostic Use.
2. All the operations must be carried out according to good laboratory practices.
3. Do not use kit components beyond the expiration date.
4. The test plate should be used immediately after opening the packaging. When it absorbs moisture, the quality deteriorates and an accurate result cannot be obtained.
5. Please do not touch the sample drop and the judgment part of the test board directly by hand.
6. Do not reuse the device.
7. The color tone of the line may vary depending on the color tone and specimen properties. However, the test result is valid as long as a red line is present.
8. If the test is invalid, one should consider the possible improper handling, inaccurate operation procedure, or device quality. Repeat the test with a new device ensuring that the test procedure has been followed accurately.
9. After use, discard the device in an infectious waste container.

STORAGE CONDITIONS

Test devices must be stored at 2~30°C. DO NOT FREEZE.

SPECIMEN HANDLING AND COLLECTION

Samples to be tested should be obtained and handled by standard microbiological procedures.

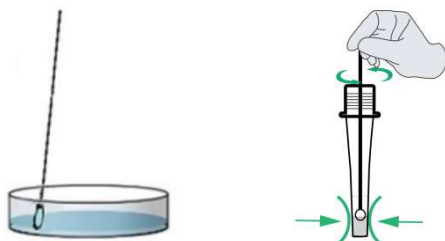
PROCEDURE

Bring the kit components to room temperature.
With bacterial colonies, we recommend the use of fresh

bacterial colonies for optimal test performance.

1. Sample Extraction

(1) From the agar culture, touch 3 colonies with a 1 µL loop, and then suspend it in the extraction tube containing the buffer. The entire bacterial colony must be suspended into the buffer.



(2) Screw the cap on the extraction tube.

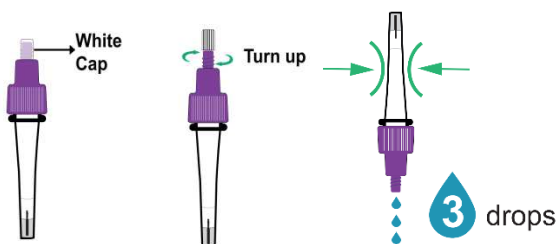
(3) Vortex to homogenize the mixture before use.



2. Test Reaction

(1) Remove test device from sealed foil pouch just prior to testing and lay flat on work bench.

(2) Open the top cap of the extraction tube, invert extraction tube and add 3 drops (80µl) of test sample into sample well by gently squeezing extraction tube.

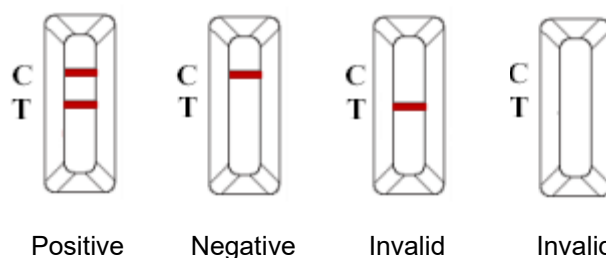


(3) Read results at 15 minutes and disregard after 30 minutes. A positive result may be visible at 3 minutes. However, the complete reaction time of 15 minutes is required to confirm a negative result.

INTERPRETATION OF RESULTS

Allow the samples to react according to the procedure and read the red purple lines that appear in the reading area.

The results are to be interpreted as follows :



Negative test result: a reddish-purple line appears across the central reading window at the Control line (C) position. No other line is present.

Positive test result: in addition to a reddish-purple band at the Control line (C), a visible reddish-purple band appears at the Test line position (T). Intensity of the test line may vary according to the quantity of antigens present in the sample. Any reddish-purple line (T), even weak, should be considered as a positive result.

Invalid test result: The absence of a Control line indicates a failure in the test procedure. Repeat the test with a new test device.

QUALITY CONTROL

Internal control

Each *EZERTM* CTX-M β-lactamases Rapid Test contains internal/procedural controls. The appearance of a control line at the Control “C” position validates the proper reagent function and assures that the correct test procedure was followed.

PERFORMANCE CHARACTERISTICS

Detection limit

The detection limit was determined using purified recombinant enzymes: 250 pg/mL

Cross-reactivity

Organisms that exhibit antibiotic resistance mechanisms were tested on CTX-M β-lactamases Rapid Test. The results showed no cross-reactivity with organisms including following antibiotic resistance mechanisms:

OXA-1, OXA-23, OXA-24, OXA-51, OXA-58, OXA-181, OXA-232, KPC-2, KPC-3, NDM-1, NDM-3, NDM-5, IMP-1, IMP-4, VIM-1, AmpC, ADC-26, ADC-154, MCR-1, SHV-1, SHV-11, SHV-53, SHV-51, SHV-105, SHV-12, SHV-123, TEM-1, aac(3)-IId, aac(3')-Ia, aac(3)-IVa, aac(6')-Ib-D181Y, rmtB, aadA3, aadA5, aadA16, arr-3, marA, marR, acrA, acrB, acrD, msbA, mdtB, mdtC, baeR, mdx, CBP-1, tet(A), tet(B), oqxA, oqxB, sul1, sul2, dfrA14, dfrA17, dfrA27, qnrS1, qnrB6, qnrA1, qnrB91, floR, PmrF, FosA6, FosA5, fosA3, fosA, catII, catB3, mecA, VanA, OmpK37, KpnG, KpnH, KpnE, KpnF.

Clinical Evaluation

(1) Performance of CTX-M β-lactamases Rapid Test was evaluated at hospital with prospectively-collected and stock bacterial isolates. The identification of CTX-M β-lactamases production on *EZERTM* CTX-M β-lactamases Rapid Test was compared to NG-Test® CTX-M Multi by

NG Biotech. A total of 178 organisms isolates were tested on *EZER*TM CTX-M β -lactamases Rapid Test with concordant results obtained by phenotypic testing paired with NG-Test[®] CTX-M Multi results.

		NG-Test [®] CTX-M		
		+	–	Total
CTX-M β -lactamases	+	135	0	135
	–	0	43	43
Rapid Test	Total	135	43	178

Relative Sensitivity: 100.00%

Relative Specificity: 100.00%

Accuracy: 100.00%

LIMITATIONS OF THE PROCEDURE

1. The performance of *EZER*TM CTX-M β -lactamases Rapid Test was established with colonies from blood agar, MacConkey agar. Performance with other culture media has not been evaluated and is therefore unknown.
2. The performance of *EZER*TM CTX-M β -lactamases Rapid Test with bacteria other than Enterobacterales has not been evaluated.
3. A negative result does not preclude the presence of CTX-M β -lactamases producing organisms.
4. A positive or a negative test does not rule out the presence of other mechanisms of antibiotic resistance.
5. This test is a qualitative assay and will not yield any quantitative result.
6. This test should be used as an aid for the rapid identification of patients bearing a resistance to CTX-M β -lactamases antibiotics.
7. The device is intended for testing on single colonies only. For positive blood culture samples, please use the *EZER*TM Blood Prime for pre-treatment.

AVAILABILITY

Product	Cat. No.	Contents
<i>EZER</i> TM CTX-M β -lactamases Rapid Test	P261104	20 Tests

REFERENCES

- [1] Mohammad Shahid, Anuradha Singh, Farrukh Sobia, Mitul Patel, & Indu Shukla. (2011). An overview of CTX-M β -lactamases. Reviews in Medical Microbiology, 22(2), 28–40.
- [2] Wei-Hua Zhao and Zhi-Qing Hu, 2013. Epidemiology and genetics of CTX-M extended-spectrum β -lactamases in Gramnegative bacteria. In: Critical Reviews in Microbiology, 2013; 39(1): 79–101

Index of Symbols

	Attention, see instructions for use		Tests per kit		Authorized Representative
	For in vitro diagnostic use only		Use by		Do not reuse
	Store between 2~30°C		Lot Number		Catalog #



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