



Cat. No.: MLT00065

For microbiology

The kit is designed to test susceptibility of Gram-negative bacteria to piperacillin/tazobactam on the basis of MIC (minimal inhibitory concentration) determination, i.e. the lowest concentration, which inhibits bacterial growth. The kit contains 36 examinations. The test is based on rehydration of antibiotics in the wells with Mueller Hinton II broth and bacterial suspension. The results are read visually after 16-20 hours of incubation.

The kit contains:

- 3 plates for examination (36 strips)
- 10 pc of PE bags
- 1 pc of storage bag (for unused strips)

Storage and expiration of the kit:

It is recommended to store the kit at (+2 to +8) °C. The date of expiration is indicated on each package. Leave plate at room temperature for at least 30 minutes before you open it to avoid water condensation.

After the aluminium package is opened and covering foil is removed, don't leave opened plates unprotected!!! Exposition to air humidity leads to antibiotic activity failure!!!

Material required to perform a test, not included in the kit:

- Sterile physiological solution
- Muller Hinton broth II cations adjusted (e.g. Suspension medium MIC, Cat.No. MLT00070)
- Sterile tubes
- Sterile basins 60 ml Cat. No. 50004457
- Frames MTD for strips Cat. No. 50004477
- A stepper or multichannel pipette for dosage of 100 µl
- A pipette for dosage of 20-60 µl
- Instrument to measure optical density of bacterial inoculum (e.g. DENSILAMETER II, Cat. No. INS00062)
- Incubator 35±2 °C
- Regular microbiological laboratory equipment (loops, marker, burner, etc.)

Caution: The kit is for professional use only! Respect the rules for work with infectious material!

Instructions for Use

Preparation of bacterial suspension (recommended procedure):

- 1) Prepare a tube with physiological solution.
- 2) Remove few colonies from 18 – 24 hour culture on blood agar and prepare a bacterial suspension of density of 0.5 on McFarland scale in physiological solution. It is important to pay special attention to the preparation of homogeneous slurry especially in *Pseudomonas*. Alternatively, a growth method: 3-5 colonies is transferred to a test tube with suitable broth and incubated to propagate to a density of 0.5 McFarland. If this density is exceeded, the suspension density adjusted sterile saline or broth to the desired value of turbidity of 0.5 McFarland.
- 3) Inoculate 60 µl of bacterial suspension into a tube with 13 ml of Suspension medium MIC and homogenise well.

Inoculation:

Remove a plate from aluminium bag and remove aluminium cover from a strip, e.g. use a scalpel or a ruler (just before inoculation). **Don't leave opened plates unprotected!!! Exposition to open air leads to antibiotic activity failure!!!** Insert any unused strips into a ZIP log storage bag. Transfer a dessicant sachet from the original package into a storage bag as well and close it for later use. Store it at 2-8°C for 4 weeks maximum.

Record number of the examined strain on the strip and insert it into a free frame. Inoculate 100 µl of suspension medium with bacterial inoculum into each well of the strip. Note: Process the strips within 60 minutes after removing it from aluminium bag.

Incubation:

Insert the inoculated strip in a frame into a PE bag. Fold the open end of the bag under the plate to prevent evaporation during the incubation. Incubate the plate at 35±2 °C for 16 – 20 hours.

Evaluation:

Remove the plate from the PE bag. To read the growth in the microwells, choose a way which is the most convenient for you:

- 1) Read against a grey background or against plate layout in the instructions.
- 2) Read against natural or artificial dispersed light.

Please read with attention:

You must see a growth in the control well (placed in row H)! If the growth is not present, the test MUST NOT be evaluated! The MIC is the lowest concentration of antibiotic in a well where no visible growth of the organism is observed. Beware to differentiate grains of growth from media bubbles. Record the results.

Tab. 1: Plate layout: piperacillin/tazobactam dilution series (in mg/l)

A	PIT 128	PIT 128	PIT 128	PIT 128	PIT 128	PIT 128	PIT 128	PIT 128	PIT 128	PIT 128	PIT 128	PIT 128
B	PIT 64	PIT 64	PIT 64	PIT 64	PIT 64	PIT 64	PIT 64	PIT 64	PIT 64	PIT 64	PIT 64	PIT 64
C	PIT 32	PIT 32	PIT 32	PIT 32	PIT 32	PIT 32	PIT 32	PIT 32	PIT 32	PIT 32	PIT 32	PIT 32
D	PIT 16	PIT 16	PIT 16	PIT 16	PIT 16	PIT 16	PIT 16	PIT 16	PIT 16	PIT 16	PIT 16	PIT 16
E	PIT 8	PIT 8	PIT 8	PIT 8	PIT 8	PIT 8	PIT 8	PIT 8	PIT 8	PIT 8	PIT 8	PIT 8
F	PIT 4	PIT 4	PIT 4	PIT 4	PIT 4	PIT 4	PIT 4	PIT 4	PIT 4	PIT 4	PIT 4	PIT 4
G	PIT 2	PIT 2	PIT 2	PIT 2	PIT 2	PIT 2	PIT 2	PIT 2	PIT 2	PIT 2	PIT 2	PIT 2
H	Growth control	Growth control	Growth control	Growth control	Growth control	Growth control	Growth control	Growth control	Growth control	Growth control	Growth control	Growth control
	Piperacillin/ Tazo- bactam 1	Piperacillin/ Tazo- bactam 2	Piperacillin/ Tazo- bactam 3	Piperacillin/ Tazo- bactam 4	Piperacillin/ Tazo- bactam 5	Piperacillin/ Tazo- bactam 6	Piperacillin/ Tazo- bactam 7	Piperacillin/ Tazo- bactam 8	Piperacillin/ Tazo- bactam 9	Piperacillin/ Tazo- bactam 10	Piperacillin/ Tazo- bactam 11	Piperacillin/ Tazo- bactam 12

Tab. 2: Clinical MIC breakpoints (mg/l) for piperacillin/tazobactam

	EUCAST			CLSI		
	susceptible	intermediate	resistant	susceptible	intermediate	resistant
<i>Enterobacteriaceae</i>	≤ 8/4	16/4*	≥ 32/4	≤ 16/4	32/4-64/4	≥ 128/4
<i>Pseudomonas spp. (P. aeruginosa CLSI)</i>	≤ 16/4		≥ 32/4	≤ 16/4	32/4-64/4	≥ 128/4
<i>Acinetobacter spp.</i>	IE		IE	≤ 16/4	32/4-64/4	≥ 128/4

*ATU (Areas of Technical Uncertainty) - before interpretation of results:

- Repeat the test
- Use an alternative test
- Downgrade the susceptibility category
- Include the uncertainty as part of the report

More on www.eucast.org

Interpretation:

The tested strain is categorized as susceptible-intermediate-resistant to piperacillin/tazobactam on the basis of MIC determination according to EUCAST interpretation tables (1) or according to CLSI document M100 (2). IE - indicates that there is insufficient evidence that *Acinetobacter spp.* is a good target for therapy with piperacillin/tazobactam. An MIC with a comment but without an accompanying S, I or R categorisation may be reported.

Other interpretative criteria must be used depending on national and laboratory standards, e.g. EUCAST Expert rules (3) or CLSI dokument M100 (2) and M07 (4). It is necessary to take following parameters into consideration when interpreting results: species identification, sample origin, patient case history, or results of additional tests.

Quality control:

We recommend following control strains for internal testing of functionality of the antibiotics in the laboratory. Follow EUCAST or CLSI standards when evaluating results.

CCM 3954 (ATCC 25922) <i>Escherichia coli</i> MIC (mg/l)	CCM 3955 (ATCC 27853) <i>Pseudomonas aeruginosa</i> MIC (mg/l)	CCM 4225 (ATCC 35218) <i>Escherichia coli</i> MIC (mg/l)
PIT 1/4-4/4	PIT 1/4-8/4	PIT 0.5/4-2/4

ATCC – American Type Culture Collection

CCM – Czech Collection of Microorganisms, Masaryk University, Faculty of Science, Kamenice 5, building A25, 625 00 Brno, tel. 549 491 430, fax 549 498 289, <http://www.sci.muni.cz/ccm>, e-mail: ccm@sci.muni.cz

Health protection:

Components of the kit are not classified as dangerous.

Disposal of the used material:

Insert the used plate into the vessel intended for the infectious material and autoclave or destroy it by incineration.

Literature:

- 1) The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MIC and zone diameters, <http://www.eucast.org>
- 2) CLSI: Performance Standards for Antimicrobial Susceptibility Testing. CLSI dokument M100. Wayne, PA: Clinical and Laboratory Standards Institute.
- 3) EUCAST expert rules in antimicrobial susceptibility testing; <http://www.eucast.org>
- 4) CLSI: Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. CLSI dokument M07. Wayne, PA: Clinical and Laboratory Standards Institute; 2015

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USED SYMBOLS



Catalogue number



In vitro diagnostics



Manufacturer



See instruction for use



Lot number



Storage temperature



Expiry date