

# MIC-Strip Piperacillin-Tazobactam



Only for *in vitro* diagnostic

english

Revision A (August 2018)

MIC-Strip for manual susceptibility testing of Gram-negative bacteria. For antibiotic composition of the strip, evaluation and interpretation of the results please refer to the evaluation protocol.

## TEST PRINCIPLE

Susceptibility testing with MIC-Strip Piperacillin-Tazobactam is based on the rehydration of the antibiotic/inhibitor combination Piperacillin-Tazobactam by adding a standardized bacteria suspension. After incubation of 18 - 22 hours at 35 – 37 °C the result is read visually and interpreted.

## REAGENTS

### Contents

40 susceptibility tests can be performed with one packaging unit. The kit contains:

- 5 plates MIC-Strip
- each plate include 8 strips with 12 wells per strip
- Cover plate and frame
- Knife

### Required additional reagents and materials:

- Mueller Hinton Broth, cation adjusted (CAMHB) (# M/E2-331-100, M/E2-331-020)
- NaCl 0,9% (# M/E2-312-001)
- Air-tight container to create a humid atmosphere

### For manual inoculation:

8-channel pipette (100-1200µl)  
 (# M/BH3-880-001 or other) incl. pipet tips  
 (# M/LH-B791204 or # M/BH3-487-096)  
 (All products with article no. are available at sifin diagnostics gmbh.)

### Laboratory materials

- McFarland standard 0,5
- Blood agar plate (without additives)
- Incubator 37 °C
- Inoculation loops
- Marking pen

## COMPOSITION OF THE MEDIA

Media	Components
NaCl 0.9% 1l	sodium chloride
Mueller Hinton Broth, cation adjusted (CAMHB) 100 tubes of 11.5 ml (± 0.5 ml) 20 tubes of 11.5 ml (± 0.5 ml)	beef extract acid hydrolysate of casein starch

### Please note:

The validity of MIC values as determined with the test system MIC-STRIP Piperacillin-Tazobactam is directly linked to the usage of the indicated Mueller Hinton Broth (M/E2-331-100, M/E2-331-020).

## STABILITY/ STORAGE

MIC-Strip plates have to be stored in the original packaging at 15 – 25 °C and can be used until the indicated expiration date. After opening of the original packaging MIC-Strips can be used for additional two months.

Mueller Hinton Broth has to be stored at 15 - 25 °C. For shelf life of the medium please follow the expiration date printed on the product.

## PRECAUTIONARY MEASURES

- Only to be used for *in vitro* diagnostic.
- Do not pipette the reagents by mouth.
- Caution in the use of the knife, risk of injury (sharp blade). After using blade push back into the protective sleeve.
- Only for proper use.
- Samples, bacteria cultures and the inoculated test plates have to be considered as potentially infectious and must be treated properly and with respect to the corresponding precautionary measures by qualified specialist staff. It is important to work aseptically during the whole test procedure. For information please refer to "BioSafety in Microbiological and Biomedical Laboratories, HHS Publication No. (CDC) 99-8395, 4th Edition (April 1999)"

or to the corresponding national legal requirements.

- Upon reading and evaluation of the tests, all samples, inoculated and contaminated products (pipette tips, stripes) must be autoclaved, burnt or disinfected in a bactericidal solution before disposal.
- It is important to follow the instructions carefully each deviation may influence the quality of the results.
- The test results should be interpreted by qualified staff with experience in microbiology. The clinical background, origin of the samples, colony and microscopic morphology, serology and the identification result must be taken into consideration when interpreting the results.

## TEST PROCEDURE

### Preparation of the samples

- Prepare a tube with 5 ml NaCl 0.9%.
- Prepare a tube of Mueller Hinton Broth.
- Pick several single colonies of an 18 - 24 hours aged pure culture from the blood agar (without additives).

### Preparation of the inoculum

- Homogenize the colonies well in 5 ml NaCl 0.9% until the turbidity matches a McFarland of 0.5.

### Mueller Hinton Broth

- Gram-negative bacteria:  
Pipette 50 µl of the bacteria suspension into 11.5 ml Mueller Hinton Broth and homogenize well.

### Inoculation

- Remove the MIC-Strip from the packaging not more than 30 minutes before inoculation.
- Cut the protective sheet along the strips with a cutter and remove the MIC-Strip.
- Put back the remaining MIC-Strip immediately into the packaging and reseal it carefully.
- Stack the removed strips into the frame and label it.
- Inoculate the prepared suspension into MIC-Strip manually by using a pipette, 100 µl per well.

### Sealing and incubation

- After the inoculation cover the strips with the cover plate.
- Place the strips in an incubator at 35 – 37°C for 18 – 22 hours.

To avoid a drying up of the medium in the wells (especially when using ventilated incubators) incubate the system in a humid atmosphere (e. g. air-tight container with damp cloth).

### Reading

- Remove the cover plate.
- Wipe off the bottom of the strips.
- Read the strips visually and document the results in the evaluation protocol.
- Turbid = growth / positive  
clear = no growth / negative
- The growth control must be covered (cloudy) otherwise the test must be repeated.

### Evaluation

Please document the results on attached evaluation protocol. The interpretation of the results is described likewise on this protocol.

## INTERPRETATION OF THE RESULTS

### MIC value

Minimum inhibitory concentration (MIC) is defined as the lowest concentration of an antibiotic that will inhibit visible growth of a microorganism. Bacterial growth can be observed as turbidity of the test medium within or as cell sediment (nodule formation) at the bottom of the respective wells of the MIC STRIP. Prerequisite for the determination of MIC values is a positive growth control.

### Skipped wells

Occasionally skipped wells could occur within the MIC-Strips. Skipped wells appear due to lack of growth in one or more wells within an antibiotic concentration series while the wells of the next higher / lower concentration of the same antibiotic show bacterial growth.

Reasons for the occurrence of skipped wells can be complex: manifestation of heteroresistance, inhomogeneous inoculum or inoculation of the wells, cross contamination of cavities by carrying over of antibiotics during pipetting, working with mixed cultures, drops of bacterial suspension at the edge of the cavities and therefore no contact with the antibiotic, etc..

For MIC-Strip systems a single skipped well within a concentration series can be ignored. The concentration of the well from which on no further growth occurs, can be read as the MIC value. If several skipped wells occur in a concentration series the test must be repeated.

### QUALITY CONTROL

The MIC-Strip and reagents are subject to quality controls which are carried out systematically at different stages of the production. The bacteriological quality control can be carried out with the following strains.

Strains	ATCC No.	DSMZ No.
<i>E. coli</i>	ATCC 25922	DSM 1103
<i>Ps. aeruginosa</i>	ATCC 27853	DSM 1117

ATCC = American Type Culture Collection  
DSMZ = Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH  
(German Collection of Microorganisms and Cell Cultures Ltd.)

### QUALITY AND PERFORMANCE DATA

The general requirements apply to DIN EN ISO 20776-1 and following or CLSI. For monitoring the accuracy for minimal inhibitory concentrations (MICs) please refer to the acceptable limits for quality control strains according to EUCAST or CLSI.

### GUARANTEE








The quality data of the MIC-Strip have been determined by strictly following the present instruction. Divergences or alterations of the test procedure may reduce the quality of the results. Any claims for damages are excluded in this case.

### TECHNICAL REMARKS

In order to obtain best results please follow the below listed points of the instructions carefully:

- MICRONAUT-Strips are intended for single use only. Strips cannot be reused.
- Work with pure culture of blood agar (without additives) not older than 24 hours.
- Use NaCl 0.9%.
- Preheat the Mueller Hinton Broth before using it (1 hour in the incubator).
- Follow the correct McFarland 0.5 adjustment of the NaCl suspension. Homogenize the suspension sufficiently.
- Transfer the bacteria suspension on a blood agar plate (without additives) for purity control.
- Cut the protective sheet along the strips with a cutter. Take care not to damage the protective sheet of adjacent strips. Do not use strips with damaged protective sheets otherwise adverse effects may arise upon loss of drug activity.
- Follow the incubation times carefully. Do not incubate bacteria less than 18 hours.
- To avoid a drying up of the medium in the wells (especially when using ventilated incubators) incubate the system in a humid atmosphere (e. g. air-tight container with damp cloth).

## EXPLANATION OF THE SYMBOLS ON THE LABELS

	For single use only
	Number of possible tests
	Storage conditions
	Instructions for use
	Security advice on the safety data sheet
	Expiry date
	CE marking in accordance with 98/79/EC (IVDD)
<b>LOT</b>	Indication of the lot number
<b>IVD</b>	In vitro diagnostics
<b>REF</b>	Article number

## LITERATURE

- Kresken, Michael, Hafner, D. and the Study Group Bacterial Resistance of the Paul-Ehrlich-Society for Chemotherapy: Drug Resistance among Clinical Isolates of Frequently Encountered Bacterial Species in Central Europe during 1975 to 1995. *Infection* 27:2-8 (1999).
- Ingo Stock, Konstanze Machka, Arne Rodloff und Bernd Wiedemann. Qualitätssicherung und Qualitätskontrollen in der Antibiotika-Empfindlichkeitsprüfung von Bakterien mit der Mikrodilution. *Chemotherapie Journal* 10:78-91 (2001).
- Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; CLSI Document M07 11th Edition (2018).
- DIN EN ISO 20776-1 - Labormedizinische Untersuchungen und In-vitro-Diagnostika-Systeme - Empfindlichkeitsprüfung von Infektionserregern und Evaluation von Geräten zur antimikrobiellen Empfindlichkeitsprüfung Teil 1: Referenzmethode zur Testung der In-vitro-Aktivität von antimikrobiellen Substanzen gegen schnell wachsende aerobe Bakterien, die Infektionskrankheiten verursachen.
- Performance Standards for Antimicrobial Susceptibility Testing; CLSI Document M100 28th Edition (2018).
- EUCAST, Breakpoint tables for interpretation of MICs and zone diameters, Version 8.0, valid from 2018-01-01.

## MIC-Strip Piperacillin-Tazobactam - Short Instruction

