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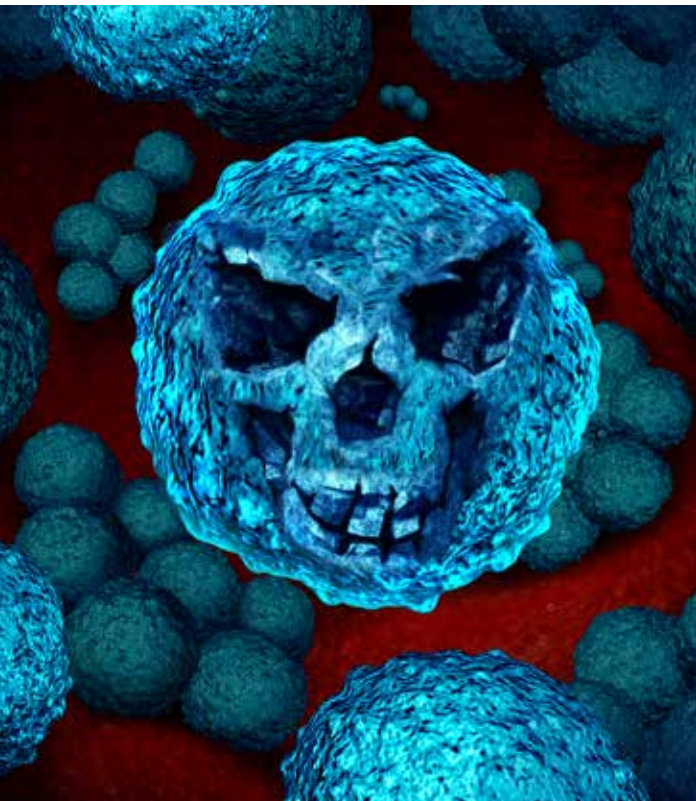
MBT STAR[®] - BL Assays

- Rapid Mass Spectrometric Resistance Detection Assays

For research use only. Not for use in clinical diagnostic procedures.

MALDI Biotyper[®]

Enabling Rapid Detection of β -lactamase Activity



The incidence of multi drug-resistant bacteria is increasing globally and is becoming an epidemic. There is an urgent need to develop accurate, reliable and cost-effective technologies for the detection of resistance mechanisms in bacteria.

Amongst the different resistance mechanisms found in bacteria, important ones are the intracellular enzymatic activities of extended-spectrum beta-lactamases (ESBLs) and carbapenemases that ultimately render antibiotics useless. These bacterial enzymes deactivate β -lactam antibiotics by hydrolysis of the β -lactam ring.

As a leading innovator, Bruker realizes the importance of microbiology research applications to aid in the detection of antibiotic resistance mechanisms. The MBT STAR-BL software module, in conjunction with the MBT STAR-Carba and MBT STAR-Cepha Kits, enables reliable, rapid and cost-effective analysis of the β -lactamase activities in bacteria.

MALDI-TOF Beyond Microorganism Identification

The fast and easy to use MBT STAR-Carba and MBT STAR-Cepha Kits offer a rapid detection of carbapenemase and cephalosporinase activity, respectively. Starting from a culture plate or a Sepsityper pellet, β -lactamase producing bacteria are detected within approximately one hour.

These mass spectrometric resistance assays extend the application of the MALDI Biotyper beyond microorganism identification.



MBT STAR-BL Assays - A Simple Workflow

To determine the level of β -lactamase activity in a bacterial isolate, samples from overnight cultures (or a Sepsityper pellet) are incubated in an antibiotic solution. After incubation, the supernatant is spotted onto a MALDI target plate and overlaid with a drop of matrix. Spectra are then acquired at the mass range of the antibiotic, using the MALDI Biotyper.

For isolates where no β -lactamase activity is present, mainly peaks corresponding to the intact antibiotic will appear in the mass spectrum. In isolates with β -lactamase activity, peaks corresponding to the hydrolyzation

products of the antibiotic will also be observed. Cleavage of the antibiotic is monitored by detection of a specific mass shift in the MALDI-TOF mass spectrum.

This fully automated monitoring is performed by the MBT STAR-BL software module, an addition to the regular MBT Compass software. An automatic calculation of the intensity of the antibiotic's peaks and corresponding ratio hydrolyzed / non-hydrolyzed antibiotic is performed. The results and corresponding report are easy to interpret by means of color codes and graphic analysis.

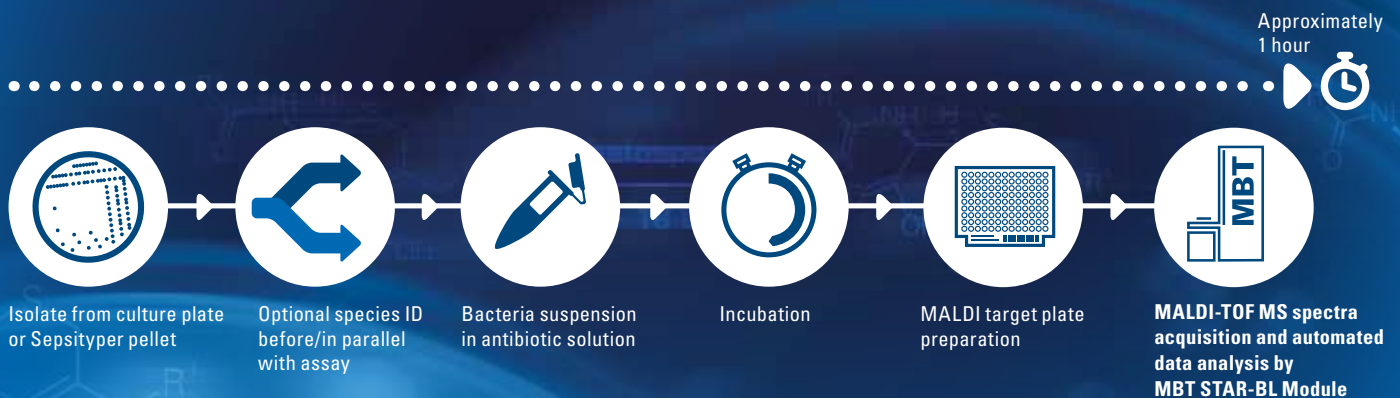


Figure 1: Fast and easy workflow of the MBT STAR-BL assays

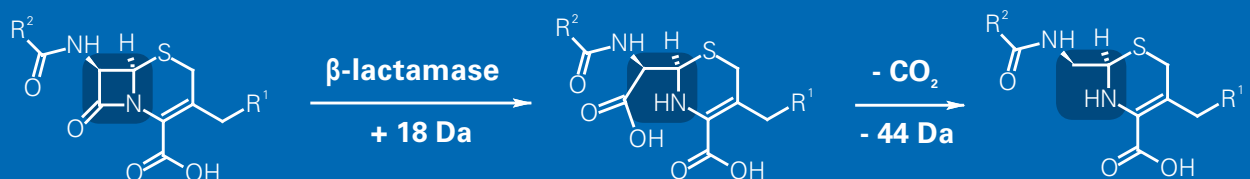


Figure 2: Hydrolysis of an antibiotic's β -lactam ring leads to mass shifts that can easily be detected by MALDI-TOF mass spectrometry

MBT STAR-Cepha Kit

For rapid detection of cephalosporinase activity towards 3rd generation cephalosporins

The MBT STAR-Cepha Kit utilizes a 3rd generation cephalosporin benchmark antibiotic, provided in convenient 96 well plates. Incubation of cephalosporinase producing bacteria with the antibiotic reagent results in cleavage (hydrolysis) of the antibiotic's β -lactam ring, hence converting the antibiotic into an inactive metabolite.

After incubation, cleavage of the benchmark antibiotic is monitored by detection of a specific mass shift in the MALDI-TOF mass spectrum.

The fully automated monitoring by the MBT STAR-BL software module results in an easy to interpret color-coded report, as shown in Figure 3.

The entire assay can be performed in less than one hour. Enterobacterales, for example, require a short incubation time of 30 minutes only.

The detection of cephalosporinase activity covers most ESBL and AmpC harboring strains, offering sensitive and selective detection of a wide range of possible cephalosporinases towards 3rd generation cephalosporins, irrespective of their genetic (pre-)characterization or prevalence:

ESBL

- e.g. plasmidic TEM-type, SHV-type and CTX-M

AmpC

- chromosomal and plasmidic
- inducible or de-repressed resistance genes
- e.g. AmpC, FOX, LAT, DHA and CMY

Run	Sample	Species	Control ID	Cepha	
190228-1403-10100201	Sample 4	<i>Klebsiella pneumoniae</i>	confirmed	0.71	H
190228-1403-10100201	Sample 5	<i>Escherichia coli</i>	confirmed	0.46	H
190228-1403-10100201	Sample 6	<i>Escherichia coli</i>	confirmed	0.56	H
	neg.control		not performed	-0.01	NH
	pos.control		not performed	1.08	H
	Δ controls			1.19	

H	Hydrolyzed ¹
NH	Non-hydrolyzed ²

Figure 3: Result report allowing easy and objective interpretation of the detection of cephalosporinase activity
¹ cephalosporinase activity detected; ² no cephalosporinase activity detected

MBT STAR-Carba Kit

For rapid detection of carbapenemase activity

The MBT STAR-Carba Kit contains a benchmark carbapenem antibiotic. Incubation of carbapenemase producing bacteria with the antibiotic reagent results in cleavage (hydrolysis) of the carbapenem's β -lactam ring, hence converting the antibiotic into an inactive metabolite. After incubation, cleavage of the carbapenem antibiotic is monitored by detection of a specific mass shift in the MALDI-TOF mass spectrum.

Detectable β -lactamase activities include Ambler class A & D serine carbapenemases (for example, KPC and OXA-type) and class B β metallo-carbapenemases (for example, VIM, NDM and IMP).

The sample can be measured after a short incubation time of only 30 to 60 minutes, dependent on the species identity and specimen type (e.g. 30 min for subcultured *Enterobacteriaceae* and *Pseudomonas* spp. and 60 min for subcultured *Acinetobacter* spp.).

The MBT-STAR-BL software module provides automated monitoring of the hydrolysis, and also enables multiple MBT-STAR assays to be performed in one run as shown in Figure 4, depicting the results for both the MBT-STAR Carba and MBT-STAR Cepha assays.

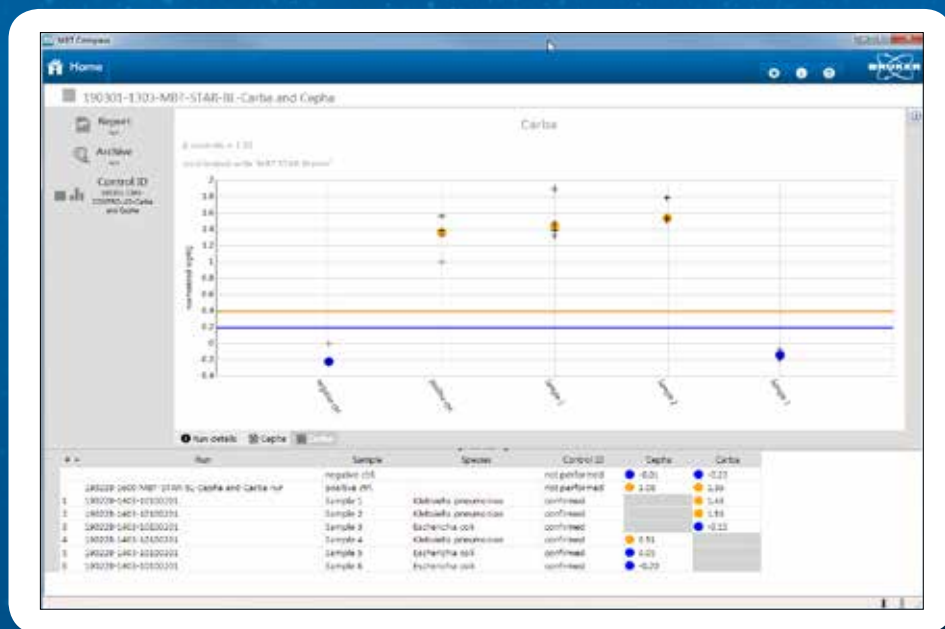


Figure 4: MBT STAR-BL software displaying a result overview of a combined MBT STAR assay run using both the MBT STAR-Cepha and MBT STAR-Carba Kit. Orange dots: cephalosporinase resp. carbapenemase activity detected; Blue dots: no cephalosporinase resp. carbapenemase activity detected

MBT STAR-BL Assay – Convenient Kits

The MBT STAR-Carba Kit and MBT STAR-Cepha Kit offer a complete solution for the sample and assay preparation, including not only the benchmark antibiotic reagent, but also a dedicated buffer, calibrant, matrix and matrix solvent, all especially designed to yield a maximum performance of the MBT STAR-BL assay.

Efficient Calibration for Accurate Analysis

Accurate analysis of intact and hydrolyzed antibiotic fragment signals in the low molecular mass range (100–1000 Da) requires an efficient calibration of the MALDI-TOF mass spectrometer.

The dedicated MBT STAR antibiotic calibration standard (MBT STAR-ACS) contains a mixture of four small peptides which provide characteristic and well-defined MALDI-TOF mass spectra signals in the mass range between 100 and 1000 Da (Figure 5).

MBT STAR-ACS is included in the MBT STAR-Carba and MBT STAR-Cepha Kits, but is also available as a separate product.

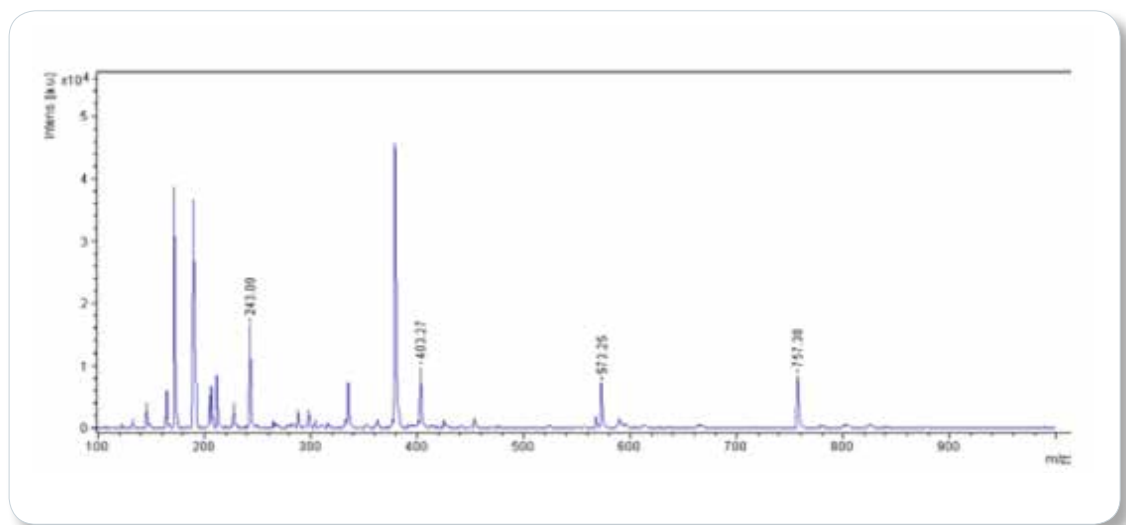


Figure 5: MALDI-TOF mass spectrum of MBT STAR Antibiotic Calibration Standard (MBT STAR-ACS)

Order Information

Pre-requisite for the MBT STAR-BL Assays is the MBT Compass software (Part No. 1843241).

Part No. 1836423 | MBT STAR-BL SW Bundle

Software for analysis of co-incubation assays for detection of β -lactamase activity in bacteria. Incl. 2 licenses to install the MBT STAR-BL software module on the acquisition system and on one additional client PC.

Part No. 1867704 | MBT STAR-Cepha Kit

The MBT STAR-Cepha Kit provides all necessary reagents and components to perform the cephalosporinase assay:

- MBT STAR Buffer
- MBT STAR Matrix
- MBT STAR Matrix Solvent
- MBT STAR-ACS Calibrator
- Water (H₂O deionized)
- MBT STAR-Cepha Antibiotic Reagent - conveniently provided in a 96 well plate



Part No. 1867701 | MBT STAR-Carba Kit

The MBT STAR-Carba Kit provides all necessary reagents and components to perform the carbapenemase assay:

- MBT STAR Buffer
- MBT STAR Matrix
- MBT STAR Matrix Solvent
- MBT STAR-ACS Calibrator
- Water (H₂O deionized)
- MBT STAR-Carba Antibiotic Reagent



Part No. 1818702 | MBT STAR-ACS

Antibiotic Calibration Standard, consisting of 5 tubes for approximately 20 calibration spots each.

Quality Control Strains

Adequate hydrolyzing (active β -lactamase expression) and respective non-hydrolyzing (no β -lactamase expression) control strains are recommended to be included within each analysis run.

Part No. 1840375 | MBT Biotarget 96

Box of twenty individually barcoded disposable 96 position MALDI target plates.

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