BRUKER

Changing Food, Water and Veterinary Microbiology

IR Biotyper®

Microbial discrimination for real-time quality control and source tracking

Innovation with Integrity

GP

Aiding quality control and source tracking in the food, water and veterinary sectors

Identifying microorganisms is a vital aspect of minimizing the risk of waterborne pathogens in water supplies, ensuring product quality in the food industry, and understanding the transmission routes of bacterial strains in veterinary samples.

Until now, the identification of bacterial outbreaks or analysis of transmission routes has required subculturing the suspect followed by monoclonal antibody techniques, pulsed-field gel electrophoresis (PFGE), multi-locus sequence typing (MLST) or whole genome sequencing (WGS). All of these approaches are associated with lengthy delays in obtaining results.

The IR Biotyper addresses this challenge by using infrared spectroscopy to classify microorganisms based on their 'molecular fingerprint', starting with colony material. This approach is fast, easy to use and economical, making it ideal for sample prescreening, strain discrimination, and cluster analysis.

Benefits of using the IR Biotyper

Implementing the IR Biotyper in your laboratory:

- Allows cluster analysis and strain discrimination on subspecies level, of bacteria and yeast found in food, water and veterinary samples

- Speeds up classification by bringing the initial screening step in-house, with same-day results starting from colony material

- Enables development of in-house screening classifiers and methods. In conjunction with the MALDI Biotyper, allows development of workflows that identify microorganisms at the genus, species and serogroup levels.
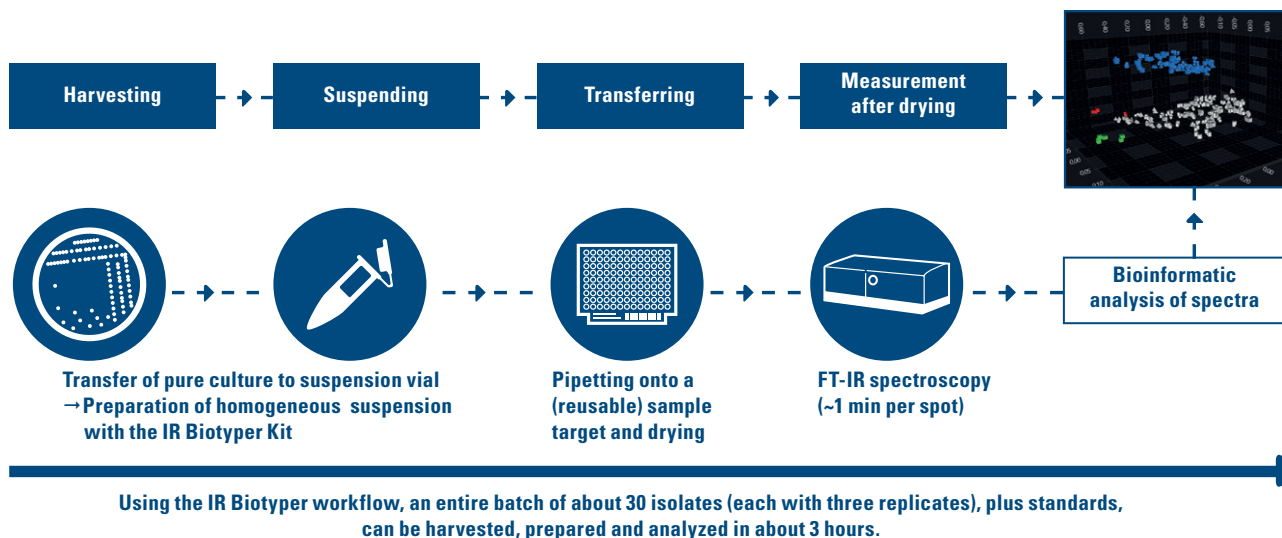
Simple workflows for rapid processing

The IR Biotyper is quick and easy to use. Starting with colony material, sample preparation onto the dedicated 96-position silicon microtiter plate takes just 30 minutes with the IR Biotyper Kit, after which the dried plate is simply inserted into the IR Biotyper.

The IR Biotyper processes an individual spot in about a minute, meaning that an entire plate can be measured in about 90 minutes. Across the whole workflow and using three replicates per isolate (plus standards), up to 30 isolates can be harvested, prepared, measured and analyzed in 3 hours.

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- Standards are part of Bruker's IR Biotyper Kit

Harvesting → **Suspending** → **Transferring** → **Measurement after drying**

Transfer of pure culture to suspension vial → Preparation of homogeneous suspension with the IR Biotyper Kit

Pipetting onto a (reusable) sample target and drying

FT-IR spectroscopy (~1 min per spot)

Bioinformatic analysis of spectra

Using the IR Biotyper workflow, an entire batch of about 30 isolates (each with three replicates), plus standards, can be harvested, prepared and analyzed in about 3 hours.

Integration with MALDI Biotyper® for genus/species-level classification

The IR Biotyper can be combined into a seamless workflow with Bruker's MALDI Biotyper, which uses MALDI-TOF MS to identify microorganisms to genus or species level within a few minutes. Data from the MALDI Biotyper can then be imported into the IR Biotyper software, to gain information on further classification such as strain discrimination or serogrouping and, once analyzed, the entire set of results can be exported to the laboratory information management system (LIMS).

The principle of FT-IR

The IR Biotyper uses Fourier transform infrared (FT-IR) spectroscopy to analyze the molecular vibrations caused by absorption of infrared light; different chemical structures vibrate at different frequencies.

FT-IR spectroscopy provides information about the full range of diagnostic biomolecules present in the cell. In routine use, the polysaccharide region is of major interest and commonly used, because this part of the spectrum provides information about the carbohydrates present in many molecules such as membrane glycoproteins, allowing microorganisms such as *Legionella pneumophila* serogroup 1, *Salmonella* O-serogroups and selected serotypes of *Listeria monocytogenes* to be classified.
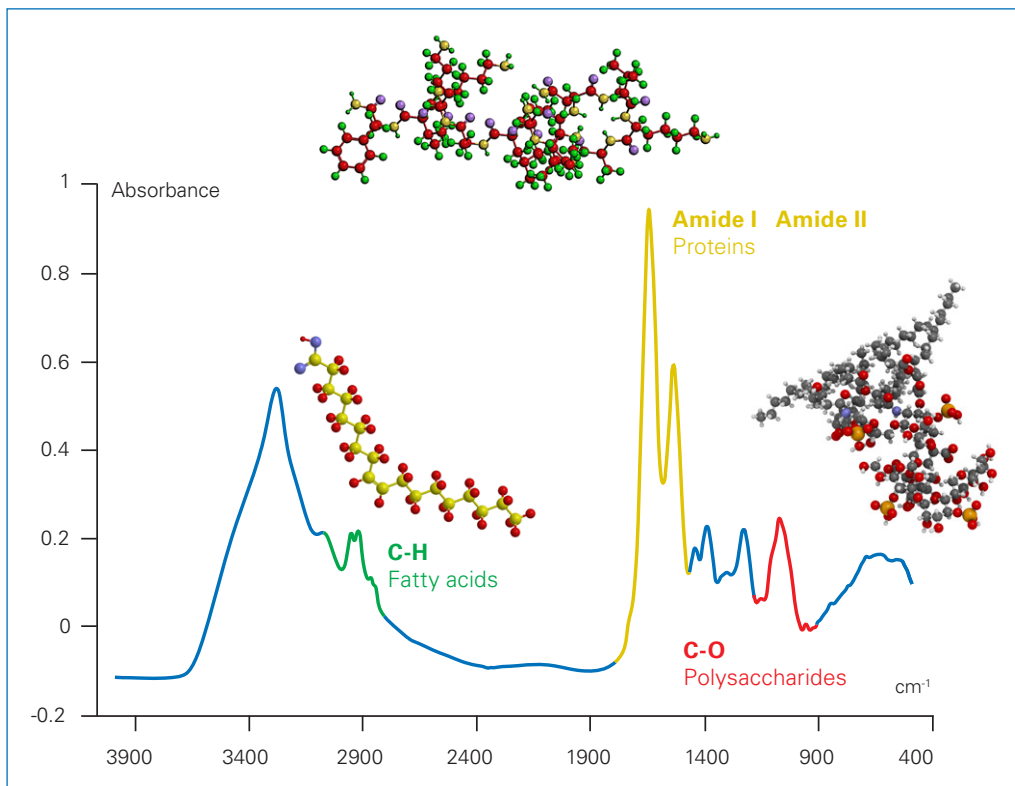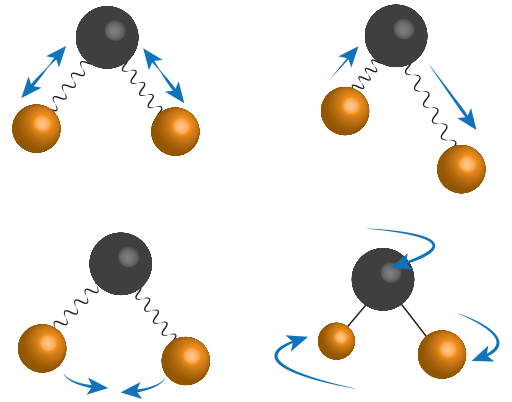
By default, the IR Biotyper analyzes IR spectra in the wavenumber range typical for polysaccharides (red), but other regions such as those indicating fatty acids (green) and proteins (yellow) can easily be selected for analysis in the software as well.
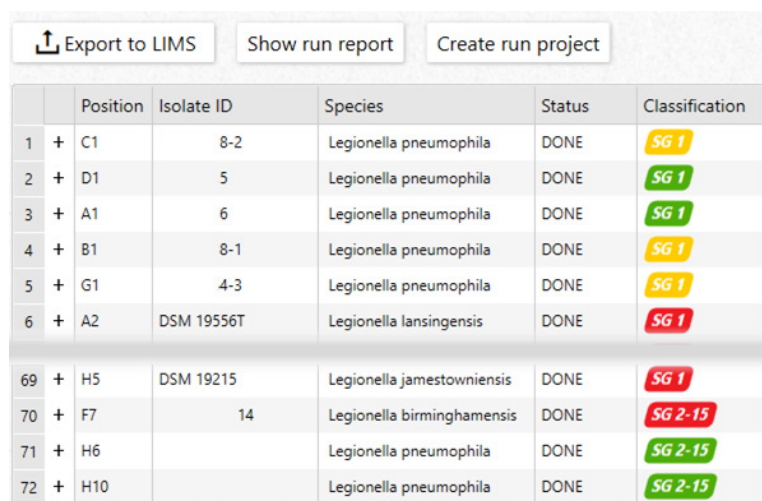
Make tedious tasks a breeze

Easy-to-use software for streamlined operation

The IR Biotyper is straightforward to use with easy-to-interpret data. Smart isolate classification is complemented by convenient visualization tools such as 2D or 3D scatter plots and straightforward export to LIMS.

Real-time, intelligent isolate classification

Classifications made by using the IR Biotyper software can be based on predefined or customized statistical models, allowing fast identification of previously identified serogroups. This classification model is based on artificial neural networks (ANN) or support vector machine (SVM) algorithms and allows users to continually build their own classification models by adding results from their dedicated applications.

		Position	Isolate ID	Species	Status	Classification
1	+	C1	8-2	Legionella pneumophila	DONE	SG 1
2	+	D1	5	Legionella pneumophila	DONE	SG 1
3	+	A1	6	Legionella pneumophila	DONE	SG 1
4	+	B1	8-1	Legionella pneumophila	DONE	SG 1
5	+	G1	4-3	Legionella pneumophila	DONE	SG 1
6	+	A2	DSM 19556T	Legionella lansingensis	DONE	SG 1
69	+	H5	DSM 19215	Legionella jamestowniensis	DONE	SG 1
70	+	F7	14	Legionella birminghamensis	DONE	SG 2-15
71	+	H6		Legionella pneumophila	DONE	SG 2-15
72	+	H10		Legionella pneumophila	DONE	SG 2-15

Software screenshot of classification of *Legionella* samples, distinguishing serogroup (SG) 1 of *L. pneumophila* from other serogroups.

Classifications rely on a score value, which is rendered in real-time using a simple 'traffic light' system for at-a-glance understanding and streamlined workflows. Finally, these results are combined in a summary report.

Customizable isolate metadata

Datasets in the IR Biotyper can be annotated with biological information (such as data from multi-locus sequence typing and pulsed-field gel electrophoresis, as well as virulence factors and resistances) or circumstantial information (such as the location, isolation date and matrix used).

Cluster analysis and strain discrimination of your samples

The IR Biotyper allows customers to analyze their own sample sets and perform cluster analysis and strain discrimination for a wide range of different species.

In sectors such as food, veterinary, and environmental, the IR Biotyper has been used to sample *E. coli, Salmonella, Citrobacter, Listeria monocytogenes, Acinetobacter baumannii, Haemophilus parasuis*, and other species.

The IR Biotyper provides a powerful tool to compare isolates in the event of outbreaks or contamination of strains of one species or multiple species. Same-day results are obtained, starting from colony material. The result of comparison of isolates can be visualized in the form of cluster analyses (dendrograms) or 3D scatter plots.

Versatile data exploration

Using the latest IR Biotyper software, results can be displayed in table format, or visualized graphically using distance matrices, dendrograms, or scatter plots.
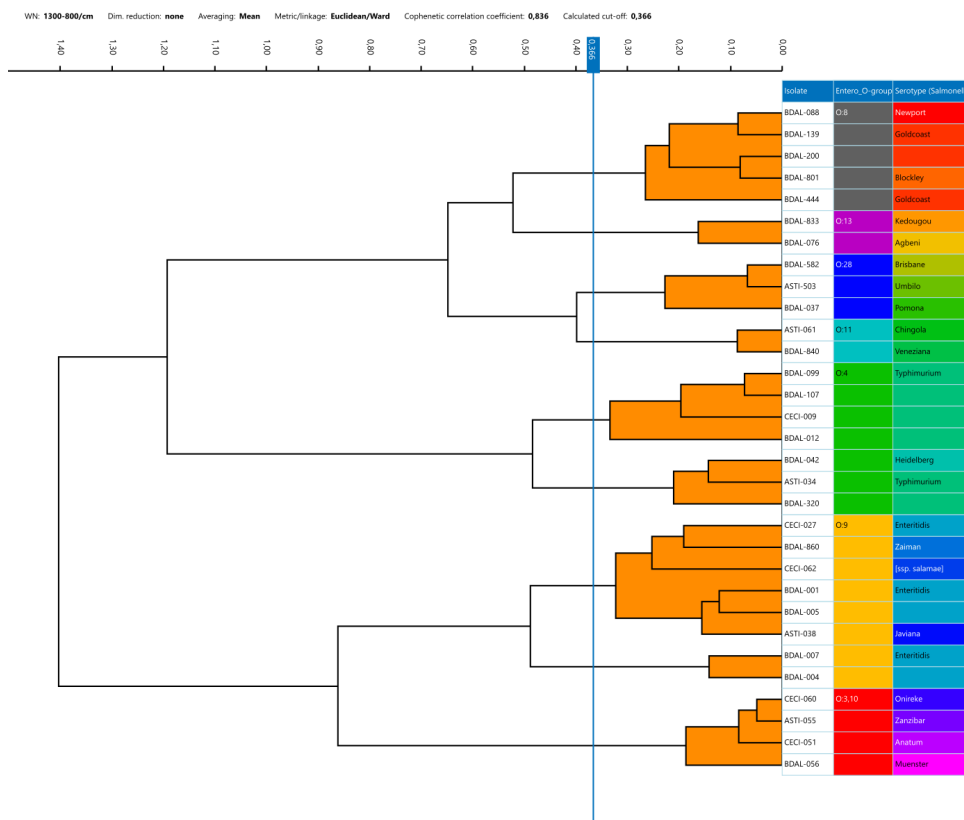

Dendrogram for clear visualization of strain discrimination for *Salmonella* O-groups and serotypes

Salmonella O-serogroups

Salmonella has over 2500 known serotypes, with about 100 accounting for most animal and human infections. Most of the latter are due to ingestion of contaminated food, especially eggs and poultry, with the risk of severe illness being high for people with weakened immune systems and children under five years old. As a result, it is important to be able to reliably identify groupings of *Salmonella*, to aid better understanding of food safety risks and animal health.

The gold-standard test, *Salmonella* agglutination, involves testing against known O- and/or H-antigens, but it is time-consuming and requires expensive consumables (which often have a limited shelf-life). Genome sequencing provides the detail on serotypes and subtypes, but is also slow and expensive, or requires sequencing staff scientists, and is not readily available to many laboratories.
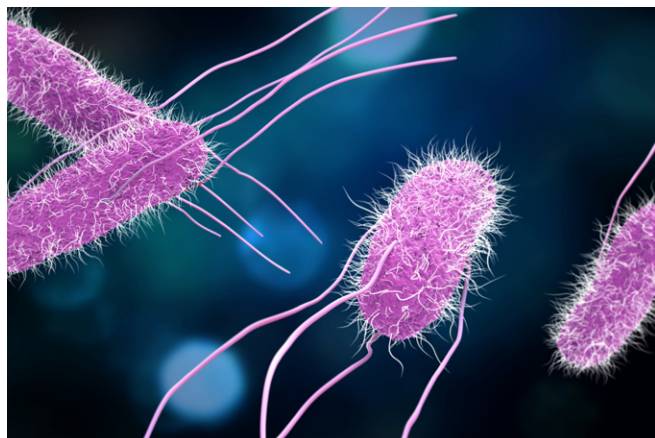
Quick and easy first-level *Salmonella* O-serogrouping

In contrast to the above-mentioned methods, the IR Biotyper offers a fast and cost-effective way of discriminating between selective *Salmonella* O-serogroups. Samples can be quickly pre-screened for the O-serogroups of greatest concern using the *Salmonella* classifier database provided with the system.

Alternatively, customized and constantly improving classification models can be developed with the help of the artificial neural network incorporated into the software. Therefore, reporting of results from *Salmonella* O-serogrouping is quick and easy using the IR Biotyper. Same-day IR Biotyper analysis is superior to other techniques and can be used as a new first level classification.

Seamless combination with MALDI Biotyper

In addition, workflows can be enhanced by adding the MALDI Biotyper for identification to genus or species level, prior to O-serogrouping using the IR Biotyper. The combination of MALDI Biotyper and IR Biotyper is therefore a valuable approach starting from colony material for food or veterinary microbiologists needing to tackle the considerable identification challenges posed by *Salmonella*.

Isolate ID	Rep.	Best result (hits)	Iso. Score
Salm 441_Dublin	3	O:9 (3)	98.0
Salm 496_Typhimurium	3	O:4 (3)	100.0
Salm 04_Paratyphi-A	3	O:2 (3)	100.0
Salm 01494_Westphalia	3	O:35 (3)	100.0
Salm 02417_Enteridis	3	O:9 (3)	98.7
Salm 84_Veneziana	3	O:11 (3)	100.0
Salm 148_Goldcoast_BO	3	O:8 (3)	100.0

Example of reporting of classification results from *Salmonella* O-serogrouping

Listeria monocytogenes

Listeria is a genus of microorganisms of which the major human pathogen is *Listeria monocytogenes*. The illness Listeriosis caused by *L. monocytogenes* is usually caused by eating contaminated food such as dairy products or fish, and primarily affects pregnant women, newborns, older adults, and people with weakened immune systems.

Serology divides *L. monocytogenes* into more than 10 serotypes on the basis of somatic and flagellar antigens. Only a small number of reference laboratories perform serology on a high level. The IR Biotyper workflow is a first level discrimination tool for the major and most important serogroups starting from colony material.

Key benefits of the IR Biotyper for *Listeria*

As part of the software package provided with the IR Biotyper, a *Listeria* classifier is supplied, enabling rapid, automated classification of the major serogroups.

Over 90% of human listeriosis cases are caused by *Listeria monocytogenes* serotypes 1/2a, 1/2b and 4b strains. As an alternative to antigen-antibody based serotyping, PCR or sequencing, a FT-IR-based method for serogrouping has been developed which provides same-day results starting from colony material. Bruker's IR Biotyper offers a rapid first-line solution for *Listeria monocytogenes* contamination source tracking – requiring only 3 hours from culture transfer to result, for 30 samples..

Isolate ID	Rep.	Best result (hits)	Iso. Score
ATCC 7644	27	1/2 (27)	🟩 100.0
CIP 105450	18	4x (18)	🟩 100.0
CIP 55.143	27	1/2 (27)	🟩 100.0
CIP 78.36	27	3 (27)	🟩 100.0
VIP 78.43	27	7 (27)	🟩 100.0
CRBIP 13.123	18	4x (18)	🟩 100.0
DSM 20600T	12	1/2 (12)	🟩 100.0
DSM 27575	12	4x (12)	🟩 100.0

Example of reporting of classification results for *Listeria* serogroups
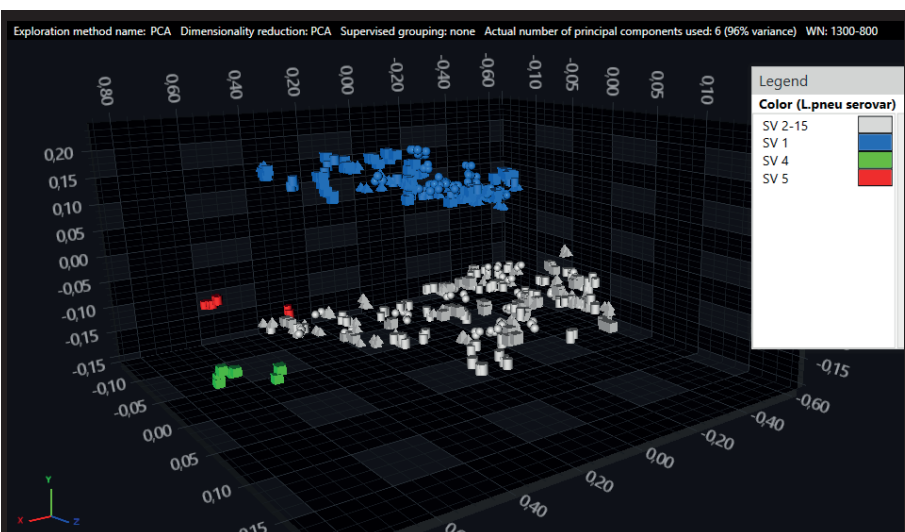
Legionella pneumophila

Since the discovery in 1979 of *Legionella pneumophila* as the causative agent of the severe pneumonia known as Legionnaires' disease, it has become one of the main concerns for regulators tasked with ensuring water safety. Most diagnostic tests are directed at serogroup 1, which poses the greatest health risk to humans.

Serogrouping of *L. pneumophila* using phenotyping methods or genotypic methods is laborious (e.g., using monoclonal antibodies or a restriction fragment analysis of plasmids and/or expensive (sequence-based typing). These drawbacks slow down the workflow or risk processing error or failure to carry out testing at all.

High sensitivity and specificity for *L. pneumophila* SG1 vs. SG 2-15 differentiation

The IR Biotyper offers an improved approach, which has been described in detail by researchers at the University of Bologna, Italy.[1] They found that the IR Biotyper allowed automatic discrimination of serogroup 1 from the other *L. pneumophila* serogroups with a sensitivity of 100% and a specificity of 98.35%, reducing laboratory costs compared to the traditional agglutination test.

This entire workflow can be completed in less than 1 hour by using the *Legionella* classifier included with the IR Biotyper software.[2] Because it is based on an artificial neural network, the analytical model can be continually improved using new data for improved classification power.

The results of a principal components analysis can be visualized using 2D or 3D scatter plots, as shown here for *Legionella pneumophila* serovars.

1. M.R. Pascale, F. Bisognin, M. Mazzotta, L. Girolamini, F. Marino, P. Dal Monte, M. Cordovana, M. Scaturro, M.L. Ricci and S. Cristino, Use of Fourier-transform infrared spectroscopy with IR Biotyper® system for *Legionella pneumophila* serogroups identification, *Frontiers in Microbiology*, 2022 (published online)
http://doi.org/10.3389/fmicb.2022.866426

2. In less than two hours from colony: *Legionella pneumophila* identification and subsequent differentiation of *L. pneumophila* serogroup 1 [Bruker News Digest #003], Bruker Daltonics, 2021
https://www.bruker.com/en/landingpages/bdal-md/news-digests-industrial-markets.html

Order information

IR Biotyper

High-performance FT-IR spectrometer
with capability for analyzing 96-spot silicon
microtiter sample plates, and updated
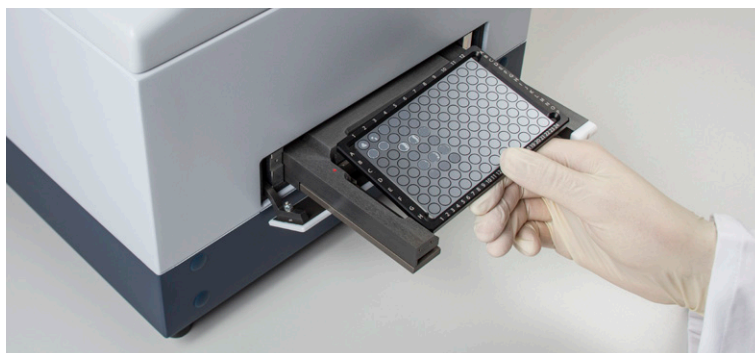software for sample setup and data analysis.

Part No. 1845471
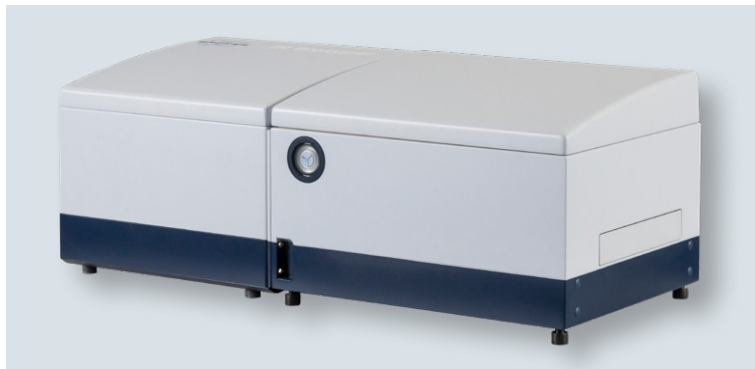
96-Spot silicon microtiter plates

Set of five reusable plates each with 96
positions designed for use with the IR
Biotyper.

Part No. I23258P

IR Biotyper Kit

Includes two bacterial IR test standards
(IRTS 1 and IRTS 2) for five runs, and sample
preparation vials for 50 isolates.

Part No. 1851760

Software package IR Biotyper 4.0

Part No. 1895589

The MALDI Biotyper and IR Biotyper can be integrated seamlessly on your lab benchtop!

The MALDI Biotyper identifies microorganisms to species level in minutes, and its data can be imported into the IR Biotyper for further strain discrimination or serogrouping.

Not for use in clinical diagnostic procedures.
Please contact your local representative for availability in your country.

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