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Name + Adresse der Firma:
Nom + adresse de l'entreprise:
Name + address of manufacturer:
Nome + indirizzo della ditta:
Nombre + dirección del fabricante:

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GenoType NTM-DR
VER 1.0

Artikelnummer (Anzahl der Teste) / numéro d'article (nombre de tests) / order no. (number of tests) / numero dell'articolo (numero del test) / número de artículo (número de tests)

29712 (12)
29796 (96)

allen anwendbaren Anforderungen der Richtlinie 98/79/EG entspricht.
remplit toutes les exigences applicables de la Directive 98/79/CE.
meets all the applicable provisions of the Directive 98/79/EC.
adempie a tutte le applicabile esigenze della Direttiva 98/79/CE.
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Normes harmonisées appliquées:
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Norme armonizzate applicate:
Normativas armonizadas aplicables:

DIN EN ISO 13485, DIN EN ISO 14971, DIN EN ISO 15223-1, DIN EN ISO 18113-1, DIN EN ISO 18113-2, DIN EN ISO 23640, DIN EN 13612, DIN EN 13641

Angewandte nationale Normen:
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May 26, 2026

Nehren, May 23, 2022

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GenoType NTM-DR

VER 1.0

Instructions for Use

IFU-297-03

CE

IVD for in vitro diagnostic use only

2019-12-18

HAIN LIFESCIENCE

GenoType **NTM-DR** VER 1.0
Molecular Genetic Assay for Detection of Resistance to Macrolides and Aminoglycosides in various Nontuberculous Mycobacterial Species (NTM) from Cultured Material

Please read the instructions on hand completely and carefully before using the kit. Strictly adhere to the established procedure to obtain correct test results.

Intended Use

The **GenoType NTM-DR** VER 1.0 is a qualitative in vitro test for detection of antibiotic resistance in members of the *Mycobacterium avium* complex, the *Mycobacterium abscessus* complex, and *Mycobacterium chelonae.*

The *M. avium* complex includes the species *M. avium, M. intracellulare*, and *M. chimaera.* The *M. abscessus* complex includes the subspecies *M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *bolletii*, and *M. abscessus* subsp. *massiliense.*

Antibiotic resistances detectable by this test are resistances to macrolides (clarithromycin, azithromycin) and aminoglycosides (kanamycin, amikacin, gentamicin). Macrolide resistance is identified (i) by the characterization of a resistance-associated part of the *erm*(41) gene (coding for an erythromycin ribosome methyltransferase only present in members of the *M. abscessus* complex) [1], and (ii) by examining the most common resistance-associated mutations of the *rrl* gene (coding for the 23S rRNA) [2].

For detection of aminoglycoside resistance, the most significant resistance-associated mutations of the *rrs* gene (coding for the 16S rRNA) [3-5] are examined.

The test is indicated as an aid for diagnosis and intended for use in medical laboratories.

Summary and Explanation

Mycobacterioses are infectious diseases caused by bacteria of the genus *Mycobacterium*. The most significant is tuberculosis (TB) caused by the members of the *Mycobacterium tuberculosis* complex.

The genus *Mycobacterium* comprises numerous species which are divided into three groups: (i) the *Mycobacterium tuberculosis* complex, (ii) *M. leprae* causing leprosy, and (iii) atypical or nontuberculous mycobacteria (NTM). In view of the varying pathogenicity and apathogenicity of some species, a fast and certain identification of the *M. tuberculosis* complex and hence its differentiation from the NTMs is most essential. NTM can cause chronic mycobacterioses. Infectiousness and symptoms vary in a broad range and depend both on the pathogen as well as on the immunocompetence of the person affected [6].

The most common mycobacterioses of patients suffering from cystic fibrosis or chronic pulmonary diseases are caused by *M. avium* and members of the *M. abscessus* complex [7] and are often poorly curable due to various resistances.

Macrolides are important components of the therapy; however, resistances may limit treatment options. One potential resistance mechanism is mediated by mutations within the *rrl* gene, particularly through substitutions of single nucleotides at positions 2058 and 2059. A mechanism of resistance that solely affects members of the *M. abscessus* complex is mediated by the gene *erm*(41). Thus, macrolide resistance arises either due to a resistance-mediating mutation in the *rrl* gene or when a functional *erm*(41) gene harbors a T at position 28. Macrolide sensitivity arises if gene *erm*(41) shows a deletion or harbors a C at position 28, provided there is no resistance-mediating mutation in the *rrl* gene. Moreover, the subspecies of *M. abscessus* complex are differentiated on the basis of the *erm*(41) gene variants described [1].

Aminoglycosides are another important element of therapy. Aminoglycoside resistance of mycobacteria is primarily caused by a modification of the ribosomal 30S subunit. For example, in *M. abscessus* subsp. *abscessus* mutations at position 1408 of the *rrs* gene lead to amikacin and kanamycin resistance.

As long as no resistance is verified, use of inadequate and hence ineffective antibiotics may lead to further spread of resistant bacteria and amplification of resistance. Therefore, rapid diagnosis and determination of the resistance status is a prerequisite for appropriate treatment.

Principles of the Procedure

The **GenoType NTM-DR** VER 1.0 is based on the **DNA•STRIP** technology. The whole procedure is divided into three steps: (i) DNA extraction from cultured material (solid/liquid medium; the necessary reagents are not included in the kit), (ii) a multiplex amplification with biotinylated primers, and (iii) a reverse hybridization.

All reagents needed for amplification, such as polymerase and primers, are included in the Amplification Mixes A and B (AM-A and AM-B) and are optimized for this test. The membrane strips are coated with specific probes complementary to the amplified nucleic acids. After chemical denaturation, the single-stranded amplicons bind to the probes (hybridization). Highly specific binding of complementary DNA strands is ensured by stringent conditions which result from the combination of buffer composition and a certain temperature. Thus, the probes reliably discriminate several sequence variations in the gene regions examined. The streptavidin-conjugated alkaline phosphatase binds to the amplicons' biotin via the streptavidin moiety. Finally, the alkaline phosphatase transforms an added substrate into a dye which becomes visible on the membrane strips as a colored precipitate. A template ensures the easy and fast interpretation of the banding pattern obtained.

Reagents and Instruments

Kit contents **Order no.** **Tests**	**29712** **12**	**29796** **96**
Kit Component 1 of 2 (store at 2°C to 8°C)		
Membrane strips coated with specific probes (NTM-DR STRIPS)	12	2x 48
Denaturation Solution (DEN) contains <2% NaOH, dye	240 µl	2x 960 µl
Hybridization Buffer (HYB) contains <10% anionic tenside, dye	12 ml	96 ml
Stringent Wash Solution (STR) contains >25% of a quaternary ammonium compound, <1% anionic tenside, dye	12 ml	96 ml
Rinse Solution (RIN) contains buffer, <1% NaCl, <1% nonionic tenside	36 ml	3x 96 ml
Conjugate Concentrate (CON-C) contains streptavidin-conjugated alkaline phosphatase, dye	120 µl	960 µl
Conjugate Buffer (CON-D) contains buffer, 1% blocking reagent, <1% NaCl	12 ml	96 ml
Substrate Concentrate (SUB-C) contains <70% dimethyl sulfoxide, <10% 4-nitro blue tetrazolium chloride, <10% 5-bromo-4-chloro-3-indolyl phosphate	120 µl	960 µl
Substrate Buffer (SUB-D) contains buffer, <1% $MgCl_2$, <1% NaCl	12 ml	96 ml
Tray, evaluation sheet	1 of each	4 of each
Instructions for use, template	1 of each	1 of each
Lot label	3	3
Kit Component 2 of 2 (store at –20°C to –18°C)		
Amplification Mix A (AM-A GT NTM-DR) contains buffer, nucleotides, Taq polymerase	120 µl	4x 240 µl
Amplification Mix B (AM-B GT NTM-DR) contains salts, specific primers, dye	420 µl	4x 840 µl

Storage, handling and disposal of kit constituents

1/2 Kit Component 1 of 2

2/2 Kit Component 2 of 2

Store all constituents from Kit Component 1 at 2°C to 8°C. Store all constituents from Kit Component 2 at –20°C to –18°C and keep strictly separated from contaminating DNA. Avoid repeated freezing and thawing of AM-A and AM-B (>4x); when processing only small sample numbers per run, aliquot AM-A and AM-B. Do not use the reagents beyond their expiry date. Dispose of unused reagents and waste in accordance with federal, state, and local regulations.

Precautions for handling kit constituents

Observe all federal, state, and local safety and environmental regulations. Always wear suitable protective clothing and gloves.
When handling kit reagents, the following special safety measures must be applied:

Hybridization Buffer **(HYB)** and Substrate Concentrate **(SUB-C)** are not classified as hazardous. Due to their ingredients, however, hazard statement EUH210 applies: Safety data sheet available on request.

Denaturation Solution **(DEN)** contains <2% sodium hydroxide.
Warning!
H315: Causes skin irritation. H319: Causes serious eye irritation.
P280: Wear protective gloves/protective clothing/eye protection. P305+351+338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P313: Get medical advice/attention.

For additional information, please refer to the safety data sheets which can be downloaded from: www.hain-lifescience.com/products/msds.html

Conjugate Concentrate **(CON-C)** and Conjugate Buffer **(CON-D)** contain biological material. Hence, they must be considered as potentially infectious and must be handled accordingly (e.g. see [8] or [9]).

Material required but not included in the kit
- Absorbent paper
- Adjustable pipettes for 10, 20, 200, and 1000 µl
- Disposable gloves
- DNA extraction kit (**GenoLyse®**, see chapter Ordering Information) as well as necessary equipment
- Disposable sterile pipette tips with filter
- Graduated cylinder
- PCR tubes, DNase- and RNase- free
- Reagents for cultivation of mycobacteria as well as necessary equipment
- Reagents for sample decontamination as well as necessary equipment
- Shaking water bath + shaking platform ***or*** **TwinCubator** (instrument for manual hybridization) ***or*** automated hybridization instrument
- Thermal cycler
- Timer
- Tweezers
- Water (distilled)
- Water (molecular biology grade, for negative controls)

Quality Control

In order to control the correct performance of the test and the proper functioning of kit constituents, each strip includes 5 control zones:
- a Conjugate Control zone (CC) to check the binding of the conjugate on the strip and a correct chromogenic reaction
- a Universal Control zone (UC) which detects all mycobacteria and members of the group of gram-positive bacteria with a high G+C content
- three Locus Control zones *(erm*(41)*, rrl, rrs)* checking the optimal sensitivity of the reaction for each of the tested gene loci

Observe the usual precautions for amplification setup. It is essential that all materials (such as pipette tips) coming in contact with the reagents are free from DNases.
Do not interchange or pool Amplification Mixes or membrane strips from different kits unless the lots are identical. You can find the kit lot and the corresponding lots of the kit constituents on the lot labels included in the kit.
A negative control sample for detection of possible contamination events containing water (molecular biology grade) instead of DNA should be part of each test run; the respective test strip should show the CC band only.

Specimen Requirements

Bacteria grown on solid medium (e.g. Loewenstein-Jensen, Middlebrook) or in liquid medium (e.g. MGIT (BD Diagnostics, Franklin Lakes, USA)) may be used as starting material for DNA extraction. Until the present edition of the instructions on hand, the performance of the test has not been validated with other sample materials.

Precautions for handling specimens
Patient specimens and cultures made from patient specimens must always be considered as infectious and must be handled accordingly (e.g. see [8] or [9]). Always wear suitable protective clothing and gloves. Samples from patients at risk (infected by pathogenic microorganisms or viruses including Hepatitis B and Human Immunodeficiency Virus (HIV)) and cultures made from those samples must always be labeled and handled under suitable safety conditions according to institutional guidelines.
All specimens that may contain mycobacteria should be handled applying Biosafety Level 2 practices or, when indicated, Biosafety Level 3 practices (e.g. see [8]). Observe all federal, state, and local safety regulations.
Discard used pipette tips immediately after use in a container for biohazardous waste. After finishing the assay, discard all used disposables in a container for biohazardous waste.

Storage and transport
All specimens should be collected and transported as recommended in the CDC publication "Public Health Mycobacteriology: A Guide for the Level III Laboratory" [10], the "Clinical Microbiology Procedures Handbook" [11], or your laboratory procedure manual.
It must be ensured that until decontamination, specimens are kept in sterile plastic containers at a temperature of 2°C to 8°C. The transport of specimens at room temperature has to be carried out as soon as possible and should be done within 1-2 days [12,13]. The specimens used for decontamination must not be older than 4 days.
After decontamination and subsequent resuspension of the bacteria pellet with phosphate buffer, samples can be stored at –80°C to –18°C for a maximum of 5 days until performing DNA extraction.

Preparation
Clinical specimens must be processed using the NALC-NaOH method according to the CDC publication "Public Health Mycobacteriology: A Guide for the Level III Laboratory" [10]. After decontamination, the cell pellet should be resuspended in a maximum of 1 to 1.5 ml of phosphate buffer. Cultivation can be performed either on solid medium (e.g. Loewenstein-Jensen, Middlebrook) or in liquid medium (e.g. MGIT (BD Diagnostics, Franklin Lakes, USA)).
Handling of potentially infectious specimens must be carried out in a class II safety cabinet.

DNA Extraction

Bacteria grown on solid medium (e.g. Loewenstein-Jensen, Middlebrook) or in liquid medium (e.g. MGIT (BD Diagnostics, Franklin Lakes, USA)) may be used as starting material for DNA extraction.
For DNA extraction, the **GenoLyse®** kit (see chapter Ordering Information) is used. For handling instructions, please refer to the respective instructions for use.
The method mentioned here was used for performance evaluation of the **GenoType NTM-DR** VER 1.0. Until the present edition of the instructions on hand, the performance of the test has not been validated with other DNA extraction methods.

Amplification

All reagents needed for amplification, such as polymerase and primers, are included in the Amplification Mixes A and B (AM-A and AM-B) and are optimized for this test. Thaw AM-A and AM-B shortly before preparing the master mix, spin down briefly, and mix carefully by pipetting up and down. Pipette AM-A and AM-B only in a room free from contaminating DNA. To avoid contamination, the DNA solution must be added in a separate working area.

Prepare for each sample:
- 10 µl AM-A (see Kit Component 2)
- 35 µl AM-B (see Kit Component 2)
- 5 µl DNA solution

Final volume: 50 µl

Determine the total number of samples (number of samples to be analyzed plus control samples). Prepare the number of tubes needed. Prepare a master mix containing AM-A and AM-B and mix carefully but thoroughly (do not vortex). Alternatively, the content of an AM-A reaction tube may completely be transferred to an AM-B reaction tube. This will lead to master mix sufficient for 12 amplification reactions (12 tests kit) or for 4x 24 amplification reactions (96 tests kit). Please note that the master mix needs to be prepared freshly each time and needs to be processed quickly. Aliquot 45 µl of the master mix into each of the prepared PCR tubes and add 5 µl water (molecular biology grade) to one aliquot (negative control sample). In a separate working area, add 5 µl DNA solution to each aliquot (except for negative control).

Amplification profile:
When using a thermal cycler from Hain Lifescience with the respective preinstallation, select protocol "MDR CUL".

15 min	95°C	1 cycle
30 sec	95°C	10 cycles
2 min	65°C	
25 sec	95°C	20 cycles
40 sec	50°C	
40 sec	70°C	
8 min	70°C	1 cycle
Heating rate		≤2.2°C/sec

Amplification products can be stored at –20°C to +8°C.

Hybridization

When using a hybridization instrument from Hain Lifescience, please refer to the document "Overview equipment programs" available on www.hain-lifescience.com for the name of the hybridization protocol to be used.
The following protocol describes the manual hybridization using a water bath or a **TwinCubator**.

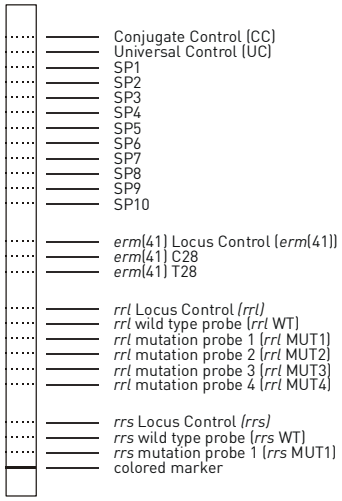
Preparation
Prewarm shaking water bath to **45°C** (the maximum tolerated deviation from the target temperature is +/–1°C) or switch on **TwinCubator**. Prewarm solutions HYB and STR to 37°C to 45°C before use. The reagents must be free from precipitates (note, however, that solution CON-D is opaque). Mix if necessary. Warm the remaining reagents with the exception of CON-C and SUB-C to room temperature. Using a suitable tube, dilute Conjugate Concentrate (CON-C, orange) and Substrate Concentrate (SUB-C, yellow) 1:100 with the respective buffer **(CON-C with CON-D, SUB-C with SUB-D)** in the amounts needed. Mix well and bring to room temperature. For each strip, add 10 µl concentrate to 1 ml of the respective buffer. Dilute CON-C before each use. Diluted SUB-C is stable for 4 weeks if stored at room temperature and protected from light.

1. **Dispense 20 µl of Denaturation Solution (DEN, blue) in a corner of each of the wells used.**
2. **Add to the solution 20 µl of amplified sample, pipette up and down to mix well and incubate at room temperature for 5 minutes.**
   Meanwhile, take strips out of the tube using tweezers and mark them with a pencil underneath the colored marker. Always wear gloves when handling strips.
3. **Carefully add to each well 1 ml of prewarmed Hybridization Buffer (HYB, green). Gently shake the tray until the solution has a homogenous color.**
   Take care not to spill solution into the neighboring wells.
4. **Place a strip in each well.**
   The strips must be completely covered by the solution and the coated side (identifiable by the colored marker near the lower end) must face upward. Using tweezers, turn over strips which might have turned when immersed in the solution. Carefully clean tweezers after each use to avoid contamination. This also applies to all following steps.
5. **Place tray in shaking water bath/TwinCubator and incubate for 30 minutes at 45°C.**
   Adjust the shaking frequency of the water bath to achieve a constant and thorough mixing of the solution. To allow adequate heat transfer, the tray must be dipped into the water to at least 1/3 of its height.
6. **Completely aspirate Hybridization Buffer.**
   For example, use a Pasteur pipette connected to a vacuum pump.
7. **Add 1 ml of Stringent Wash Solution (STR, red) to each strip and incubate for 15 minutes at 45°C in shaking water bath/TwinCubator.**

8. **Work at room temperature from this step forward.**
   Completely remove Stringent Wash Solution.
   Pour out Wash Solution in a waste container and remove all remaining fluid by turning the tray upside down and gently striking it on an absorbent paper. This also applies to all other wash steps.
9. **Wash each strip once with 1 ml of Rinse Solution (RIN) for 1 minute on shaking platform/TwinCubator (pour out RIN after incubation).**
10. **Add 1 ml of diluted Conjugate (see above) to each strip and incubate for 30 minutes on shaking platform/TwinCubator.**
11. **Remove solution and wash each strip twice for 1 minute with 1 ml of Rinse Solution (RIN) and once for 1 minute with approx. 1 ml of distilled water (e.g. use wash bottle) on shaking platform/TwinCubator (pour out solution each time).**
    Make sure to remove any trace of water after the last wash.
12. **Add 1 ml of diluted substrate (see above) to each strip and incubate protected from light without shaking.**
    Depending on the test conditions (e.g. room temperature), the substrate incubation time, i.e. the time until the bands are clearly visible, can vary between 3 and 20 minutes. Extended substrate incubation times can lead to increased background staining and might impair interpretation of the results.
13. **Stop reaction as soon as bands are clearly visible by briefly rinsing twice with distilled water.**
14. **Using tweezers, remove strips from the tray and dry them between two layers of absorbent paper.**

Evaluation and Interpretation of Results

Paste strips and store protected from light. An evaluation sheet is included in the kit. When using this evaluation sheet, paste the developed strips in the designated fields by aligning the bands CC and UC with the respective lines on the sheet. The supplied template also serves as an aid for evaluation and must be aligned with the bands CC and UC, as well as – separately for each present locus – with the respective Locus Control band. Determine the species with the help of the interpretation chart and enter the name of the identified species in the respective column. Determine the resistance status and note down in the respective column. As a help for interpretation, evaluation examples are given in the subsequent chapter. Each strip has a total of 24 reaction zones (see figure).



Note: The strip is not displayed in original size.

Conjugate Control (CC)
A line must develop in this zone, documenting the efficiency of conjugate binding and substrate reaction.

Universal Control (UC)
This zone detects all mycobacteria and members of the group of gram-positive bacteria with a high G+C content. If this zone and the Conjugate Control zone stain positive but the remaining banding pattern cannot be assigned to a specific mycobacterium, additional methods have to be applied to identify the respective bacterial species.

Species-specific probes (SP1-SP10)
Specific probes, for evaluation see interpretation chart.

Locus Controls (*erm*(41), *rrl*, and *rrs*)
The Locus Control zones detect a gene region specific for the respective locus. Since the *erm*(41) locus is only present in members of the *M. abscessus* complex, the *erm*(41) probe will only stain positive for members of this complex. In case of a positive test result (evaluable wild type or mutation banding pattern), the signals of the Locus Control bands may be weak.

***erm*(41)**
The *erm*(41) gene is examined for detection of resistance to macrolides (clarithromycin or azithromycin) and is only present in members of the *M. abscessus* complex.
The *erm*(41) C28 probe detects a genotype that carries a C at position 28 of the *erm*(41) gene. When the *erm*(41) C28 probe stains positive, this indicates that the tested strain is sensitive to macrolides (except for strains with an additional *rrl* mutation) [1].
The *erm*(41) T28 probe detects a genotype that carries a T instead of a C at position 28 of the *erm*(41) gene. When the *erm*(41) T28 probe stains positive, this indicates that the tested strain is resistant to macrolides [1].
The probes *erm*(41) C28 and *erm*(41) T28 are only relevant for *M. abscessus* subsp. *abscessus* and *M. abscessus* subsp. *bolletii*, but not for *M. abscessus* subsp. *massiliense*. Due to deletions in the *erm*(41) gene of *M. abscessus* subsp. *massiliense* the gene is nonfunctional, leading to macrolide sensitivity in spite of a developed *erm*(41) T28 band (except for strains with an additional *rrl* mutation) [1].

rrl
The *rrl* gene is examined for detection of resistance to macrolides (clarithromycin or azithromycin).
The wild type probe comprises the most important resistance region of the *rrl* gene (see table 1). When the wild type probe stains positive, there is no detectable mutation within the examined region. In case of a mutation, the respective amplicon cannot bind to the wild type probe resulting in the absence of the wild type probe signal.
The mutation probes detect the most common resistance-mediating mutations (see table 1).
Each pattern deviating from the wild type pattern (see evaluation example 1) indicates a macrolide resistance of the tested strain.

Table 1: Mutations in the *rrl* gene and the corresponding wild type and mutation bands [2]

Failing wild type band	Analyzed nucleic acid position	Developing mutation band	Mutation	Phenotypic resistance
rrl WT	2058-2059	*rrl* MUT1	A2058C	Macrolides
		rrl MUT2	A2058G	
		–	A2058T	
		rrl MUT3	A2059C	
		rrl MUT4	A2059G	
		–	A2059T	

rrs

The *rrs* gene is examined for detection of resistance to aminoglycosides (kanamycin, amikacin, gentamicin).
The wild type probe comprises the most important resistance region of the *rrs* gene (see table 2). When the wild type probe stains positive, there is no detectable mutation within the examined region. In case of a mutation, the respective amplicon cannot bind to the wild type probe resulting in the absence of the wild type probe signal.
The mutation probe detects the most common resistance-mediating mutation (see table 2).
Each pattern deviating from the wild type pattern (see evaluation example 1) indicates an aminoglycoside resistance of the tested strain.

Table 2: Mutations in the *rrs* gene and the corresponding wild type and mutation bands [3-5]

Failing wild type band	Analyzed nucleic acid position	Developing mutation band	Mutation	Phenotypic resistance
rrs WT	1406-1409	*rrs* MUT1	A1408G	Aminoglycosides
		–	T1406A	
			C1409T	

Please note:
Not all bands of a strip have to show the same signal strength. Only those bands whose intensities are about as strong as or stronger than that of the UC band are to be considered.

Identification of macrolide resistance mediated by the *erm*(41) gene is only possible for members of the *M. abscessus* complex. If the strain investigated does not belong to the *M. abscessus* complex, the entire *erm*(41) gene locus (all bands including the Locus Control band) is absent.

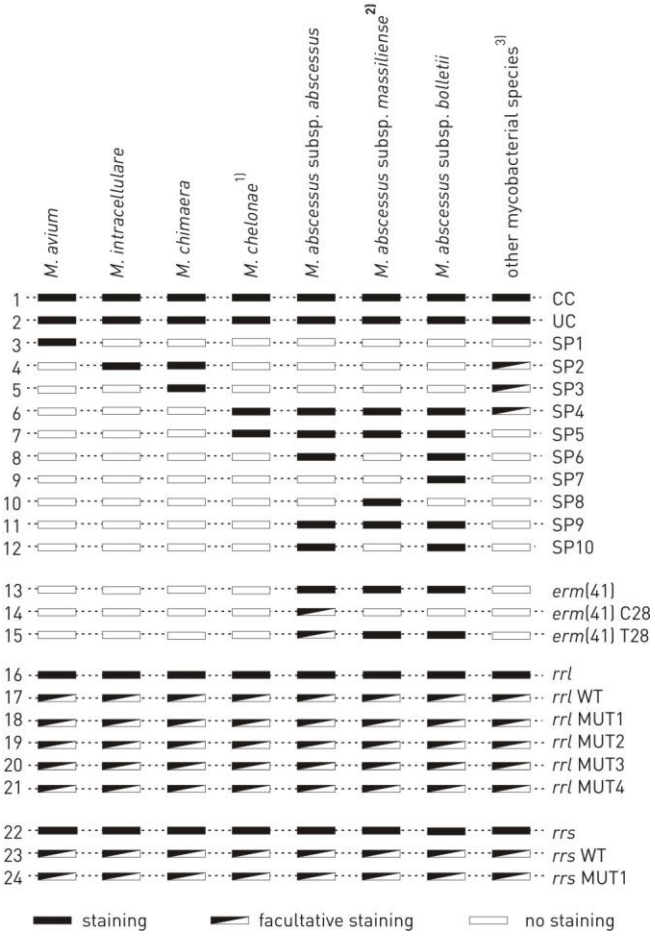
In case the entire *rrl* and/or *rrs* gene locus (all bands including the Locus Control band) is missing while at least one species-specific band (SP1-SP10) is developed, the test result is invalid.

When both a mutation probe and the corresponding wild type probe of a strip are developed, this represents a valid result. Possible reasons could be:
- The tested specimen contains a heteroresistant strain.
- The tested specimen contains more than one mycobacterial strain (e.g. due to mixed infection of the patient).

Theoretically, a resistance caused by a mutation within the examined gene regions can exist in spite of a wild type pattern. Possible reasons could be:
- The tested specimen contains a strain that has developed a heteroresistance and the resistance is caused by a mutation not covered by the mutation probes.
- The tested specimen contains a wild type and a resistant strain (e.g. due to mixed infection of the patient) and the resistance is caused by a mutation not covered by the mutation probes.

Additional mycobacterial species can be identified with the **GenoType Mycobacterium CM** and the **GenoType Mycobacterium AS**.

Interpretation Chart



Band No. 1 (CC): Conjugate Control
Band No. 2 (UC): Universal Control

1) *M. immunogenum* shows the same banding pattern as *M. chelonae*.
2) Please note the special criteria for resistance detection in *M. abscessus* subsp. *massiliense* (see evaluation examples 4 and 5).
3) Species may be identified with the **GenoType Mycobacterium CM** and the **GenoType Mycobacterium AS**.

Evaluation Examples

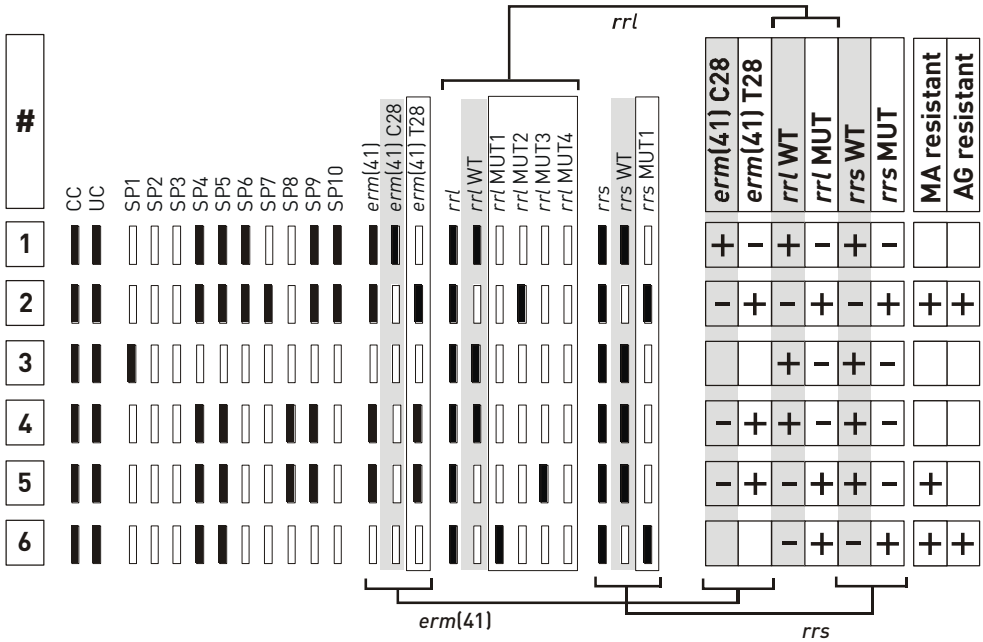


Figure 1: Examples for banding patterns and their evaluation with respect to macrolide (MA) and/or aminoglycoside (AG) resistance

If the wild type band displays a signal, this is classified as positive and marked in the WT column of the respective gene as "+". If the wild type band is absent, this is classified as negative and marked in the WT column as "–". Negative entries are only made to the mutation columns when none of the mutation bands displays a coloration. If at least one of the mutation bands displays a coloration, this is classified as positive and the MUT column of the respective gene is marked with a "+". To the resistance columns a "+" is assigned only if at least one entry in the WT and MUT columns or the "*erm*(41) C28" and "*erm*(41) T28" columns deviates from the wild type pattern of the respective gene (see example 1).

Below, the examples shown above are explicated:

Example 1 shows the wild type banding pattern of *M. abscessus* subsp. *abscessus*. All wild type probes and "*erm*(41) C28" but none of the mutation probes display a signal; hence, the evaluation chart shows a "+" in the "*erm*(41) C28" column and in the two wild type columns and a "–" in the "*erm*(41) T28" column and in the two mutation columns. Accordingly, no entry is made in the resistance columns.

Example 2 shows a possible banding pattern of *M. abscessus* subsp. *bolletii*. The "*erm*(41) C28" band is missing and the "*erm*(41) T28" band is developed. Hence, the evaluation chart shows a "-" in the "*erm*(41) C28" column and a "+" in the "*erm*(41) T28" column. The *rrl* wild type band is missing, and the mutation band "*rrl* MUT2" is developed; hence, the field in the "*rrl* WT" column is marked with a "–", the field in the "*rrl* MUT" column is marked with a "+", and the strain is evaluated as resistant to macrolides. Finally, the *rrs* wild type band is missing and the mutation band "*rrs* MUT1" is developed; hence, the field in the "*rrs* WT" column is marked with a "–", the field in the "*rrs* MUT" column is marked with a "+"; hence, an aminoglycoside resistance is detected.

Example 3 shows the wild type banding pattern of *M. avium*. The *erm*(41) locus is missing as is correct; hence, no entries are made in the respective columns. The *rrl* locus and the *rrs* locus display the wild type banding pattern resulting in a wild type entry as in example 1. The strain is evaluated as sensitive to macrolides and aminoglycosides.

Example 4 shows a possible banding pattern of *M. abscessus* subsp. *massiliense*. The "*erm*(41) C28" band is missing and the "*erm*(41) T28" band is developed. Hence, the evaluation chart shows a "-" in the "*erm*(41) C28" column and a "+" in the "*erm*(41) T28" column. Since the probes erm(41) C28 and erm(41) T28 are, however, not relevant for the evaluation of a phenotypic resistance to macrolides in *M. abscessus* subsp. *massiliense* (see chapter Evaluation and Interpretation of Results), there must be an additional mutation in the *rrl* gene to evaluate the strain as resistant to macrolides. Here, the *rrl* wild type band is present, and no mutation band is developed; hence, the field in the "*rrl* WT" column is marked with a "+", the field in the "*rrl* MUT" column is marked with a "-", and the strain is evaluated as sensitive to macrolides. The *rrs* locus displays the wild type banding pattern which is depicted accordingly.

Example 5 shows a possible banding pattern of *M. abscessus* subsp. *massiliense*. The "*erm*(41) C28" band is missing and the "*erm*(41) T28" band is developed. Hence, the evaluation chart shows a "-" in the "*erm*(41) C28" column and a "+" in the "*erm*(41) T28" column. Since the probes erm(41) C28 and erm(41) T28 are, however, not relevant for the evaluation of a phenotypic resistance to macrolides in *M. abscessus* subsp. *massiliense* (see chapter Evaluation and Interpretation of Results), there must be an additional mutation of the *rrl* gene to evaluate the strain as resistant to macrolides. Here, the *rrl* wild type band is actually missing, and the mutation band "*rrl* MUT3" is developed; hence, the field in the "*rrl* WT" column is marked with a "–", the field in the "*rrl* MUT" column is marked with a "+", and the strain is evaluated as resistant to macrolides. The *rrs* locus displays the wild type banding pattern which is depicted accordingly.

Example 6 shows a possible banding pattern of *M. chelonae* or *M. immunogenum*. The *erm*(41) locus is missing as is correct; hence, no entries are made to the respective columns. The *rrl* wild type band is missing and the mutation band "*rrl* MUT1" is developed; hence, the field in the "*rrl* WT" column is marked with a "–", the field in the "*rrl* MUT" column is marked with a "+", and the tested strain is evaluated as resistant to macrolides. Finally, the *rrs* wild type band is missing and the mutation band "*rrs* MUT1" is developed; hence, the field in the "*rrs* WT" column is marked with a "–", the field in the "*rrs* MUT" column is marked with a "+", and an aminoglycoside resistance is detected.

Limitations

Strictly adhere to the established protocols and procedures in order to obtain correct test results and to avoid contaminations.
Use of this assay is limited to qualified personnel well trained in the test procedure and familiar with molecular biological methods.
The test reflects the current state of knowledge of Hain Lifescience.

As any DNA-based assay, this test only screens the nucleic acid sequence and not the amino acid sequence. Therefore, it is possible that mutations in the probe region that do not cause an amino acid exchange (silent mutations) will still produce the absence of one of the wild type bands.
The **GenoType NTM-DR** VER 1.0 only detects those resistances that have their origins in the *erm*(41), *rrl*, and *rrs* regions examined here. Resistances originating from mutations of other genes or gene regions as well as other macrolide and aminoglycoside resistance mechanisms will not be detected by this test.

The presence of multiple bacterial species in the sample to be analyzed might hamper the interpretation of the test.

The test only works within the limits of the genomic regions the primers and probes were chosen from.
As any detection system based on hybridization, the test system on hand bears the possibility that sequence variations in the genomic regions the primers and probes were chosen from but the detection of which the test was not designed for may lead to false results. Due to the high variability of bacterial genomes, it is possible that certain subtypes might not be detected.

Performance evaluation of this assay was carried out with the **GenoLyse®** kit for DNA extraction from cultured material. Until the present edition of the instructions on hand, the performance of the test has not been validated with other DNA extraction methods or sample materials.

The results of this test may only be interpreted in combination with additional laboratory and clinical data available to the responsible physician. In addition, results of phenotypic drug susceptibility testing have to be considered in certain cases.
The user must have or acquire information about the local mutation distribution pattern of the genes investigated with this test. Confirmation of the test results by phenotypic drug susceptibility testing may be necessary.

Troubleshooting

Overall weak or no signals (including Conjugate Control zone)

- Room temperature too low or reagents not equilibrated to room temperature.
- No or too little amount of CON-C and/or SUB-C used.
  Repeat reverse hybridization.

Weak or no signals except for Conjugate Control zone

- Quality of extracted DNA does not allow an efficient amplification. Repeat extraction.
- Amplification Mixes (AM-A and AM-B) were not mixed properly, interchanged, or added in wrong amounts. Prepare a new master mix and repeat amplification.
- Incubation temperature too high. Repeat reverse hybridization.

No homogeneous staining

- Strips were not completely immersed during incubation steps.
- Tray was not shaken properly.
  Repeat reverse hybridization.

High background color

- CON-C and/or SUB-C used too concentrated.
- Washing steps were not performed with the necessary care.
- Wash solutions too cold.
  Repeat reverse hybridization.

Unexpected result

- Wrong incubation temperature.
- Hybridization Buffer and/or Stringent Wash Solution were not properly prewarmed or mixed.
- Contamination of neighboring wells by spillage during addition of Hybridization Buffer.
  Repeat reverse hybridization.
- Contamination of extracted DNA with previously extracted or amplified DNA. Repeat extraction.
- Contamination of amplification reagents. In this case, a negative control sample shows additional bands besides CC and AC. Repeat amplification using fresh reagents.
- Depending on the amount of amplified DNA used and on the specific reaction conditions, a strong and fast color development may occur. In such cases, discontinue the substrate incubation as soon as the signals are clearly visible in order to prevent the development of cross-hybridizing bands.
- No pure culture as starting material. Re-culture in order to exclude contamination.
- Improper sampling, storage, transport, or preparation of specimen. Request a new specimen and repeat test.
- Error during DNA extraction. Repeat extraction.

Ordering Information

	Order no.
GenoType NTM-DR VER 1.0 (kit for analysis of 12 samples)	29712
GenoType NTM-DR VER 1.0 (kit for analysis of 96 samples)	29796
GenoLyse® (kit for manual DNA extraction of 12 samples)	51612
GenoLyse® (kit for manual DNA extraction of 96 samples)	51610

Performance Characteristics

For performance evaluation of the **GenoType NTM-DR** VER 1.0 the test was carried out according to the instructions on hand.

Diagnostic performance

Diagnostic performance characteristics of the **GenoType NTM-DR** VER 1.0 were determined in a study with 96 samples. The test panel consisted of 88 strains identifiable with the **GenoType NTM-DR** VER 1.0 test kit (covering all species identifiable with the test system), and 8 strains not identifiable with the test kit. DNA extraction from cultured material was performed with the **GenoLyse®** kit according to the respective instructions for use. For evaluation of species identification, all samples were characterized by the **GenoType NTM-DR** VER 1.0, by sequencing, and by the **GenoType Mycobacterium CM** VER 1.0. One sample contained a mixed culture of two identifiable strains. This sample was excluded from evaluation. The other 95 isolates showed correct results. For evaluation of resistance detection, the **GenoType NTM-DR** VER 1.0 was compared to phenotypic drug susceptibility testing (DST). All 87 isolates identifiable with the kit showed correct results.

Table 1: Performance characteristics of the **GenoType NTM-DR** VER 1.0 for species identification from cultured material compared to sequencing/ **GenoType Mycobacterium CM** VER 1.0 (GT Myco CM)

		Sequencing/ GT Myco CM		
		positive	negative	Diagnostic sensitivity: 100%
				Diagnostic specificity: 100%
GenoType NTM-DR VER 1.0	positive	87	0	Positive predictive value: 100%
	negative	0	8	Negative predictive value: 100%

Table 2: Performance characteristics of the **GenoType NTM-DR** VER 1.0 for detection of clarithromycin resistance from cultured material compared to DST

		DST		
		resistant	sensitive	Diagnostic sensitivity: 100%
				Diagnostic specificity: 100%
GenoType NTM-DR VER 1.0	resistant	20	0	Positive predictive value: 100%
	sensitive	0	67	Negative predictive value: 100%

Table 3: Performance characteristics of the **GenoType NTM-DR** VER 1.0 for detection of amikacin/tobramycin resistance from cultured material compared to DST

		DST		
		resistant	sensitive	Diagnostic sensitivity: 100%
				Diagnostic specificity: 100%
GenoType NTM-DR VER 1.0	resistant	5	0	Positive predictive value: 100%
	sensitive	0	82	Negative predictive value: 100%

Analytical performance

Analytical specificity

The specificity of this test is ensured by the accurate design of specific primers and probes which considers, among others, homology comparisons of the sequences published in gene databases, and by stringent reaction conditions.

The analytical specificity of the **GenoType NTM-DR** VER 1.0 was determined with DNA isolates of the following *Mycobacterium* species identifiable by this test:

M. abscessus subsp. *abscessus, M. abscessus* subsp. *bolletii*, *M. chelonae, M. chimaera, M. immunogenum, M. intracellulare, M. abscessus* subsp. *massiliense.*

Furthermore, the following strains not detectable with the test system were analyzed:

Actinomyces naeslundii, Aggregatibacter actinomycetemcomitans, Bacillus cereus, Bordetella pertussis, Corynebacterium amycolatum, C. jeikeium, C. minutissimum, C. spec., *C. ulcerans, Escherichia coli, Gordona rubropertinctus, Haemophilus influenzae, Klebsiella oxytoca, K. pneumoniae, Mycobacterium africanum, M. alvei, M. asiaticum, M. bovis BCG, M. bovis* subsp. *bovis, M. canettii, M. celatum, M. fortuitum, M. frederiksbergense, M. gastri, M. genavense, M. goodie, M. gordonae, M. haemophilum, M. heckeshornense, M. interjectum, M. intermedium, M. kansasii, M. lentiflavum, M. mageritense, M. malmoense, M. marinum, M. mucogenicum, M. palustre, M. peregrinum, M. pinnipedii, M. scrofulaceum, M. shimoidei, M. simiae, M. smegmatis, M. szulgai, M. triplex, M. tuberculosis, M. ulcerans, M. xenopi,* MRSA, *Nocardia amarae, N. asteroides, N. brasiliensis, N. farcinica, N.* spec., *Porphyromonas gingivalis, Prevotella intermedia, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pneumoniae, Tannerella forsythia, Treponema denticola, Tsukamurella inchonensis, T. pulmonis.*

All species identifiable with the test were correctly identified. All other isolates not detectable with the test system displayed no specific banding pattern for species identification and no evaluable banding pattern for macrolide and aminoglycoside resistances. Hence, an analytical specificity of 100% was achieved.

Analytical sensitivity (limit of detection, LOD)

Analytical sensitivity of the **GenoType NTM-DR** VER 1.0 was determined using culture dilutions of *M. abscessus* subsp. *abscessus*. Each culture dilution was prepared in 20 parallels. DNA was extracted using the **GenoLyse®** kit and analyzed with the **GenoType NTM-DR** VER 1.0 applying the "MDR CUL" PCR protocol. A limit of detection (LOD; lowest bacterial concentration that generates a positive test result with a confidence of 95%) of 3.3x 10^4 bacteria/ml was determined.

Reproducibility

Intra-assay precision

In order to determine the intra-assay precision of the **GenoType NTM-DR** VER 1.0, two M. *abscessus* subsp. *abscessus* culture dilutions (3.3x 10^6 and 3.3x 10^4 bacteria/ml, respectively), one *M. szulgai* culture dilution and one negative control were set up in triplicate and tested under identical conditions with the **GenoType NTM-DR** VER 1.0. DNA extraction was performed using the **GenoLyse®** DNA extraction kit. All strains showed the expected signals and the negative controls were negative. No deviations were detected within the parallels, the banding patterns were identical and the signals strengths were comparable. Hence, the intra-assay precision was 100%.

Inter-assay precision

In order to determine the inter-assay precision of the **GenoType NTM-DR** VER 1.0, the same samples as described for the intra-assay precision were applied. These samples and a negative control were tested in nine runs: on three different days, using three different sets of instruments, and conducted by three different operators. DNA extraction was performed using the **GenoLyse®** DNA extraction kit. Apart from the varied parameter, all other testing conditions were identical. No deviations were detected between parallel samples, between runs banding patterns were identical and correct and signal strengths were comparable. Hence, the inter-assay precision was 100%.

Interfering substances
There are substances that may inhibit PCR reactions. Such inhibitors may, for example, originate from the culture medium. In order to assess if the medium influences the **GenoType NTM-DR** VER 1.0, 6 different samples were cultured in 4 different media (solid media: Loewenstein-Jensen, Stonebrink, and Middlebrook-7H10; liquid medium: MGIT (BD Diagnostics, Franklin Lakes, USA)). DNA was extracted using the **GenoLyse®** DNA extraction kit and then tested with the **GenoType NTM-DR** VER 1.0.
All samples showed the same correct results. Hence, it can be excluded that the tested media import inhibitors into the **GenoType NTM-DR** VER 1.0.

Stability
Shelf life of the test kit when stored as recommended: see box label.
Stability is determined according to DIN EN ISO 23640.

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Important Changes in IFU-297-03

Chapter	Change
Reagents and Instruments	The former chapters "Kit Contents", "Storage and Disposal of Kit Constituents", "Precautions for Handling Kit Constituents", and "Material Required but not Included in the Kit" are now subchapters of the new heading "Reagents and Instruments".
Evaluation Chart	**New:** *"M. immunogenum* shows the same banding pattern as *M. chelonae."*

CE IVD

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DE
EN

Konformitätserklärung
Declaration of Conformity

Wir Hain Lifescience GmbH
We (Name des Anbieters / Supplier´s name)

Hain Lifescience GmbH
Hardwiesenstraße 1
72147 Nehren
Germany

(Name und Anschrift des Herstellers Anschrift / Manufacturer's name and address)

erklären in alleiniger Verantwortung, dass das Produkt
declare under our sole responsibility that the product

GenoLyse® VER 1.0

REF: 51612 (12 tests), 51610 (96 tests)
Basic-UDI: 40490550300001057UL
Risikoklasse: A
Risk Class:
Zweckbestimmung: Der GenoLyse® VER 1.0 ist ein Kit für die manuelle Isolierung von bakterieller DNA zur anschließenden Verwendung in diagnostischen Verfahren. Das Produkt ist ein In-vitro-Diagnostikum zur Anwendung von Gesundheitsfachkräften in klinischen Laboratorien.

Intended Purpose: The GenoLyse® VER 1.0 is a kit for manual extraction of bacterial DNA for subsequent use in diagnostic procedures. The device is an in vitro diagnostic product for use by healthcare professionals in clinical laboratories.

(Bezeichnung, Typ oder Modell, Basic UDI-DI, Risikoklasse, Zweckbestimmung /
Name, type or model, Basic UDI-DI, classification, Intended Purpose)

auf das sich diese Erklärung bezieht, mit der/den folgenden Norm(en) oder normative Dokument(en) übereinstimmt:
to which this declaration relates is in conformity with the following standard(s) or other normative document(s):

DIN EN ISO 13485
DIN EN ISO 14971
DIN EN ISO 15223-1
DIN EN ISO 18113-1
DIN EN ISO 18113-2
DIN EN 23640
DIN EN 13612
DIN EN 62366

(Titel und/oder Nr. sowie Ausgabedatum der Norm(en) oder der anderen normativen Dokumente /
Title and/or number and date of issue of the standard(s) or other normative document(s))

Gemäß den Bestimmungen der Verordnungen und Gemeinsamen Spezifikationen
Following the provisions of Regulation(s) and Common specifications

Annex II and III of Regulation EU 2017/746 (IVDR)

Benannte Stelle (n/a für Produkte der Risikoklasse A- nicht steril)
Notified Body (n/A for products of risk class A – non sterile)

N/A – Class A Non-sterile

Nehren, 2022-06-24
(Ort und Datum der Ausstellung / Place and date of issue)

Guy Francis
General Management

Gültig bis: 2025-06-23
Valid until

Stefanie Hammoud-Schütt
Director Regulatory Affairs (PRRC)

(Name und Unterschrift oder gleichwertige Kennzeichnung des Befugten / Name and signature or equivalent marking of authorized person)

Diese Konformitätserklärung entspricht der Europäischen Norm EN ISO/IEC 17050-1 »Konformitätsbewertung - Konformitätserklärung von Anbietern - Teil 1: Allgemeine Anforderungen«

This Declaration of Conformity fulfils the European Standard EN ISO/IEC 17050-1 » Conformity assessment - Supplier's declaration of conformity - Part 1: General requirements«.

Anhänge sind Bestandteil dieser Erklärung. Diese Erklärung bescheinigt die Übereinstimmung mit den genannten Verordnungen, beinhaltet jedoch keine Zusicherung von Eigenschaften. Die Sicherheitshinweise der mitgelieferten Produktdokumentation sind zu beachten.

Appendices are part of this declaration. This declaration certifies the conformance with the stated regulations, however warranty of characteristics is not included. The safety instructions contained in the product documentation must be observed.

GenoLyse®

VER 1.0

Instructions for Use

IFU-51610-16

CE

IVD for in vitro diagnostic use only

2023-03-06

Regulatory notices

Note:
If any serious incident has occurred in relation to the device this shall be reported to the manufacturer and to the competent authority of the user's localization. Please use the following mail address:
info.mdx.de@bruker.com

Document history

Title:	Instructions for Use **GenoLyse**® VER 1.0
Revision:	Revision 16 (March 06, 2023)
First release:	May 07, 2008

Important changes in IFU-51610-15

Chapter	Change
	Generally revised and restructured: New index: "Table of Contents" The former chapters "Material Required but not Included in the Kit" and "Ordering Information" are now combined in the subchapter "Materials required but not included in the kit". The chapter "Storage and disposal of kit constituents" was restructured and is presented in tabulation. The chapter "Quality Control" was restructured into the subchapters "General measures", "Internal control", and "External controls". The chapter "Procedure" was restructured into the subchapters "Preparation of samples", "DNA extraction for use with downstream applications without an Internal Control" and "DNA extraction for use with downstream applications with an Internal Control". The chapter "Troubleshooting" was restructured and is presented in tabulation. New chapter: "Symbols"
Intended Purpose	The former chapter Intended Use was renamed to Intended Purpose and adapted to the requirements of the IVD-R.
Preparations	All information on preparation of samples were moved to the instructions for use of the test kits.

Important changes in IFU-51610-16

Chapter	Change
	General information: Information about regulatory notices added.
Important changes	Former chapter "Important changes" is now a subheading under the new heading "Document history".

Contents

GenoLyse® VER 1.0
Kit for extraction of bacterial DNA

Please read the instructions on hand completely and carefully before using the kit. Strictly adhere to the established procedure to obtain correct test results.

1 Intended purpose

The **GenoLyse®** VER 1.0 is a kit for manual extraction of bacterial DNA for subsequent use in diagnostic procedures. The device is an in vitro diagnostic product for use by healthcare professionals in clinical laboratories.

2 Principles of the procedure

The **GenoLyse®** VER 1.0 permits manual extraction of bacterial DNA. The extraction of DNA comprises the following three steps: (i) pelleting of cells for removal of sample liquids, (ii) lysis under alkaline conditions at elevated temperature, and (iii) neutralization.

3 Reagents and instruments

3.1 Kit contents

	Σ 12	Σ 96
REF	**51612**	**51610**
Lysis Buffer (A-LYS) contains 0.5-<1% nonionic tenside, <0.2% NaOH, dye	1.2 ml	9.6 ml
Neutralization Buffer (A-NB) contains buffer	1.2 ml	9.6 ml
Instructions for use	1	1

3.2 Storage and disposal of kit constituents

Kit constituent	Storage area	Storage temperature
A-LYS	DNA extraction	2°C to 8°C
A-NB	DNA extraction	2°C to 8°C

Do not use damaged kit components.
Do not use the reagents beyond their expiry date.
Dispose of unused reagents and waste in accordance with federal, state, and local regulations.

3.3 Precautions for handling kit constituents

Observe all federal, state, and local safety and environmental regulations. Always wear suitable protective clothing, protective gloves, and eye protection.

For additional information on the hazardous substances included in the kit, please refer to the safety data sheets which can be downloaded from: www.hain-lifescience.com/products/msds.html.

3.4 Materials required but not included in the kit

Table 1
General and specific equipment

Product	Description	Available from	REF
Disposable gloves			
Pipettes	Calibrated, adjustable (10 to 1000 µl)		
Sterile pipette tips	With filter, free from DNases		
Tabletop centrifuge	For 1.5 ml reaction tubes		
Vortexer			
Water bath	Precision +/- 1°C		
Timer			
1.5 ml screw cap tubes	With skirted base, with assembled cap, sterile	Sarstedt, Nümbrecht, Germany	72.692.005

For additionally required materials for DNA extraction, please refer to the instructions for use of the respective downstream application.

4 Quality control

4.1 General measures

Observe the usual precautions for nucleic acid extraction. It is essential that all materials (such as pipette tips) coming in contact with the reagents are free from DNases.

4.2 Internal control

It may be necessary to add an Internal Control DNA during nucleic acid extraction. Please refer to the instructions for use of the respective downstream application.

4.3 External controls

The preparation of negative and positive control samples is described in the instructions for use of the respective downstream application.

5 Specimen requirements

The applicable starting materials for the test kit used are stated in the respective instructions for use.

5.1 Precautions for handling specimens

Patient specimens and cultures made from patient specimens must always be considered as infectious and must be handled accordingly (e.g. see [1] or [2]).
All specimens that may contain mycobacteria should be handled applying Biosafety Level 2 practices or, when indicated, Biosafety Level 3 practices (e.g. see [1]). Observe all federal, state, and local safety regulations.
Discard used pipette tips immediately after use in a container for biohazardous waste. After finishing the assay, discard all used disposables in a container for biohazardous waste.

5.2 Storage and transport

The applicable starting materials for the downstream application are stated in the respective instructions for use. Observe the given instructions for sampling, storage, transport, and preparation of the specimens and, when indicated, special precautions for handling.

6 Procedure

Please note:

The working area must be free from contaminating DNA.

6.1 Preparation of samples

For information on the preparation of samples for suitable downstream applications, please refer to the respective instructions for use.

6.2 DNA extraction for use with downstream applications without an Internal Control

1. **Determine the number of samples** (number of samples to be analyzed plus control samples).
2. **Centrifuge prepared samples** (for information on sample preparation, please refer to the instructions for use of the respective downstream application) **for 15 min at 10,000 x g in a standard tabletop centrifuge and discard supernatant.**
3. **Resuspend the pellet in 100 µl A-LYS by vortexing.**
4. **If a negative control sample for detection of possible contamination events shall be included:**

   Transfer 100 µl A-LYS to an empty sample tube (see chapter 3.4).

   The negative control sample is processed like regular samples.
5. **Incubate closed sample tube for 5 min at 95°C in a water bath and subsequently spin down briefly.**
6. **Add 100 µl Neutralization Buffer (A-NB) and vortex sample for 5 s.**
7. **Spin down for 5 min at full speed** (at least 10,000 x g) **in a tabletop centrifuge and directly use 5 µl of the supernatant for PCR.**
   In case the DNA solution is to be stored, transfer the supernatant to a new tube and store it at or below –18°C.

6.3 DNA extraction for use with downstream applications with an Internal Control

1. **Determine the number of samples** (number of samples to be analyzed plus control samples).
2. **Centrifuge prepared samples** (for information on sample preparation, please refer to the instructions for use of the respective downstream application) **for 15 min at 10,000 x g in a standard tabletop centrifuge and discard supernatant.**
3. **Thaw Internal Control DNA** (included in the downstream application) **and equilibrate to room temperature** (15°C to 25°C)**, vortex for 5 s, and spin down briefly.**
4. **Prepare a mix of 2 µl IC and 100 µl Lysis Buffer** (A-LYS) **per sample and close tube properly. Mix thoroughly by vortexing** (5 s to 10 s). Refreeze IC immediately after use.
5. **Resuspend the pellet in 100 µl A-LYS/IC mix by vortexing.**
6. **If a negative control sample for detection of possible contamination events shall be included:**

   Transfer 100 µl A-LYS/IC mix to an empty sample tube (see chapter 3.4).

   The negative control sample is processed like regular samples.
7. **Incubate closed sample tube for 5 min at 95°C in a water bath and subsequently spin down briefly.**
8. **Add 100 µl Neutralization Buffer (A-NB) and vortex sample for 5 s.**
9. **Spin down for 5 min at full speed** (at least 10,000 x g) **in a tabletop centrifuge and directly use 5 µl of the supernatant for PCR.**
   In case the DNA solution is to be stored, transfer the supernatant to a new tube and store it at or below –18°C.

7 Limitations

- Strictly adhere to the established protocols and procedures in order to obtain correct test results and to avoid contaminations.
- Use of this kit is limited to qualified personnel well trained in the procedure and familiar with molecular biological methods.
- The results generated with DNA extracted with this kit may only be interpreted in conjunction with additional laboratory and clinical data available to the responsible physician.
- The performance evaluation of the **GenoLyse®** VER 1.0 was carried out with compatible downstream applications, applying the conditions indicated in the respective instructions for use. The starting materials included in the respective instructions for use were tested. For performance data of the **GenoLyse®** VER 1.0 in combination with compatible downstream applications, please refer to the respective instructions for use.

8 Troubleshooting

8.1 Problems in subsequent applications

Observation	Possible cause	Recommendation
No valid results can be generated.	Inhibition due to interfering substances	Use only the intended sample material.
	DNA solution contains protein contaminations.	Repeat DNA extraction.
	Improper sampling, storage, transport, or preparation of specimen.	Request a new specimen and repeat DNA extraction.
Contamination of extraction reagents.	In the subsequent application, species-specific DNA is also detected in a negative control included in the DNA extraction.	Exchange the extraction reagents. Request new specimens and repeat extraction.
Unexpected result	Improper sampling, storage, transport, or preparation of specimen.	Request new specimen and repeat extraction.

9 Performance characteristics

For performance data of the **GenoLyse**® VER 1.0 in combination with compatible downstream applications from Hain Lifescience, please refer to the respective instructions for use.

9.1 Stability

Shelf life of the kit when stored as recommended: see box label.
Stability is determined according to DIN EN ISO 23640.

10 References

1. Biosafety in microbiological and biomedical laboratories, 5th edition. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, USA 2009.
2. Protection of laboratory workers from occupationally acquired infections. Approved guideline. Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards), USA, Document M29 (please refer to the latest version).

11 Symbols

11.1 Harmonized symbols

Symbol	Meaning	Symbol	Meaning
CE	CE mark	UDI	Unique device identifier
IVD	In vitro diagnostic medical device	REF	Catalog number
Σ <n>	Sufficient for <n> assays		Use by
	Manufacturer	LOT	Batch code
	Consult instructions for use		Date of manufacture
	Caution		Temperature limitations

CE IVD

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ZERTIFIKAT ◆ CERTIFICATE ◆ 認證證書 ◆ СЕРТИФИКАТ ◆ CERTIFICADO ◆ CERTIFICAT

DAkkS Deutsche Akkreditierungsstelle D-ZM-11321-01-00

TÜV SÜD Product Service

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GERMANY

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Valid until: 2027-08-17

Date, 2024-08-12

Christoph Dicks
Head of Certification/Notified Body

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No. Q5 079456 0006 Rev. 02

Applied Standard(s):

ISO 13485:2016
(EN ISO 13485:2016/AC:2018, EN ISO 13485:2016/A11:2021)
Medical devices - Quality management systems - Requirements for regulatory purposes

Facility(ies):

Hain Lifescience GmbH
Hardwiesenstr. 1, 72147 Nehren, GERMANY

Administration, Quality Management, Technical Service, Design and Development and Manufacturing of reagents, instruments and software, Quality Control, Warehousing and Distribution of molecular biological in-vitro diagnostics reagents for Clinical Chemistry, Immunology, Infectious Diseases and Genetic Disorders.
Installation and Maintenance services for molecular biological in-vitro diagnostic instruments for Infectious Diseases and Genetic Disorders.

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Manufacturing of molecular biological in-vitro diagnostic reagents for Clinical Chemistry, Immunology, Infectious Diseases and Genetic Disorders.

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Warehousing, Marketing, Distribution and IT of molecular biological in-vitro diagnostic Reagents and Instruments for Clinical Chemistry, Immunology, Infectious Diseases and Genetic Disorders.
The provision of Warehousing and Distribution services for molecular biological in-vitro diagnostic instruments for Infectious Diseases and Genetic Disorders.

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