GenoLyse®

VER 1.0

Instructions for Use

IFU-51610-14

CE

IVD for in vitro diagnostic use only

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GenoLyse®
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.

Intended Use

The **GenoLyse®** DNA extraction kit permits the fast and easy manual extraction of bacterial DNA for further use with the following diagnostic assays from Hain Lifescience: **GenoQuick® CT**, **GenoQuick® MTB**, **GenoType CM*direct***, **GenoType MTBC**, **GenoType MTBDR*plus***, **GenoType MTBDR*sl***, **GenoType Mycobacterium AS**, **GenoType Mycobacterium CM**, and **GenoType NTM-DR**. Depending on the subsequent test, patient specimens and/or cultured material can be used as starting material.
The kit is an in vitro diagnostic product for use in medical laboratories.

Principles of the Procedure

The whole procedure is divided into three steps: (i) pelleting of cells for removal of sample liquids, (ii) lysis under alkaline conditions at elevated temperature, and (iii) neutralization. The extracted DNA may directly be used for downstream applications or can be stored at –20°C.

Storage and disposal of kit constituents

Store all kit components at 2°C to 8°C. 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.
For additional information, please refer to the safety data sheets which can be downloaded from: www.hain-lifescience.com/products/msds.html

Specimen Requirements

The applicable starting materials for the diagnostic test kit (**GenoQuick® CT**, **GenoQuick® MTB**, **GenoType CM*direct***, **GenoType MTBC**, **GenoType MTBDR*plus***, **GenoType MTBDR*sl***, **GenoType Mycobacterium AS**, **GenoType Mycobacterium CM**, or **GenoType NTM-DR**) are stated in the respective instructions for use. Observe the given instructions for storage, transport, and preparation of the specimens and, when indicated, special precautions for handling.

Precautions for handling specimens
Patient specimens and cultures made from patient specimens must always be considered as potentially infectious and must be handled accordingly (e.g. see [1] or [2]). Always wear suitable protective clothing and gloves. Samples from patients at risk (infected by pathogenic microorganisms 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.
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.

Quality Control

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.
For detection of possible contamination events a negative control sample should be included in the sample set during DNA extraction. The preparation of negative controls is described in the chapter Procedure.

Procedure

A. For use with the GenoQuick® MTB, GenoType MTBC, GenoType MTBDR*plus*, GenoType MTBDR*sl*, GenoType Mycobacterium CM VER 1.0, **or GenoType NTM-DR assay:**
Handling of potentially infectious specimens must be carried out in a class II safety cabinet. Potentially infectious samples must be centrifuged in a class II safety cabinet or in an aerosol-tight rotor. Open aerosol-tight rotor in safety cabinet only. For inactivated samples, a standard rotor can be used for centrifugation outside the safety cabinet.

If a negative control sample for detection of possible contamination events shall be included in DNA extraction, pipette 100 µl Lysis Buffer (A-LYS) into a 1.5 ml screw cap tube. For further processing the negative control, proceed with step 4.

1. When using patient specimens (only **GenoQuick® MTB**, **GenoType MTBDR*plus***, and **GenoType MTBDR*sl***), transfer 500 µl of decontaminated sample material into a labeled 1.5 ml screw cap tube; when using bacteria grown in liquid medium (only **GenoType MTBC**, **GenoType MTBDR*plus***, **GenoType MTBDR*sl***, **GenoType Mycobacterium CM** VER 1.0, and **GenoType NTM-DR**), transfer 1 ml.
   When using bacteria grown on solid medium (only **GenoType MTBC**, **GenoType MTBDR*plus***, **GenoType MTBDR*sl***, **GenoType Mycobacterium CM** VER 1.0, and **GenoType NTM-DR**), collect bacteria with an inoculation loop and suspend in 100 µl of Lysis Buffer (A-LYS), vortex, and continue with step 4.
2. Centrifuge for 15 min at 10,000 x g.
3. Discard supernatant and resuspend pellet in 100 µl Lysis Buffer (A-LYS) by vortexing.
4. Incubate sample for 5 min at 95°C in a water bath. Briefly spin down.
5. Add 100 µl Neutralization Buffer (A-NB) and vortex sample for 5 sec.
6. Spin down for 5 min at full speed in a table top centrifuge and directly use 5 µl of the supernatant for PCR.
   In case the DNA solution is to be stored for an extended period of time, transfer supernatant to a new tube.

The extracted DNA may directly be used for downstream applications or can be stored at –20°C.

B. For use with the GenoQuick® CT assay:
If a negative control sample for detection of possible contamination events shall be included in DNA extraction, pipette 100 µl Lysis Buffer (A-LYS) into a 1.5 ml screw cap tube. For further processing the negative control, proceed with step 4.

1. When using swabs with transport medium, rinse out swab in transport medium by vortexing for 10 seconds. Squeeze out any residual liquid at the inner wall of the tube. Discard swab.
   When using dry swabs, rinse out swab in 0.5-1 ml 0.9% NaCl solution by vortexing for 10 seconds. Squeeze out any residual liquid at the inner wall of the tube. Discard swab.
2. Transfer 500 µl of sample material from step 1 or 500 µl of first void urine into a labeled 1.5 ml screw cap tube. Centrifuge for 15 min at 10,000 x g in a standard table top centrifuge.
3. Discard supernatant and resuspend pellet in 100 µl Lysis Buffer (A-LYS) by vortexing.
4. Incubate sample for 5 min at 95°C in a water bath. Briefly spin down.
5. Add 100 µl Neutralization Buffer (A-NB) and vortex sample for 5 sec.
6. Directly use 5 µl of the DNA solution for PCR.
   In case the DNA solution is to be stored for an extended time period, spin down for 5 min at full speed and transfer supernatant to a new tube.

The extracted DNA may directly be used for downstream applications or can be stored at –20°C.

C. For use with the GenoType CM*direct*, GenoType Mycobacterium AS, or GenoType Mycobacterium CM VER 2.0 **assay:**
Handling of potentially infectious specimens must be carried out in a class II safety cabinet. Potentially infectious samples must be centrifuged in a class II safety cabinet or in an aerosol-tight rotor. Open aerosol-tight rotor in safety cabinet only. For inactivated samples, a standard rotor can be used for centrifugation outside the safety cabinet.

Determine the number of samples (number of samples to be analyzed plus negative control sample). Prepare an A-LYS/IC mix containing 100 µl Lysis Buffer (A-LYS) and 2 µl Internal Control DNA (IC GT CMdirect for the **GenoType CM*direct***, IC GT Mycobacterium AS for the **GenoType Mycobacterium AS**, or IC GT Mycobacterium CM VER 2.0 for the **GenoType Mycobacterium CM** VER 2.0; included in the respective **GenoType** kit) for each sample. Mix the A-LYS/IC mix thoroughly (vortex).

If a negative control sample for detection of possible contamination events shall be included in DNA extraction, pipette 100 µl A-LYS/IC mix into a 1.5 ml screw cap tube. For further processing the negative control, proceed with step 4.

1. When using patient specimens (only **GenoType CM*direct***), transfer 500 µl of decontaminated sample material into a labeled 1.5 ml screw cap tube; when using bacteria grown in liquid medium (only **GenoType Mycobacterium AS** and **GenoType Mycobacterium CM** VER 2.0), transfer 1 ml.
   When using bacteria grown on solid medium (only **GenoType Mycobacterium AS** and **GenoType Mycobacterium CM** VER 2.0), collect bacteria with an inoculation loop and suspend in 100 µl A-LYS/IC mix, vortex, and continue with step 4.
2. Centrifuge for 15 min at 10,000 x g.
3. Discard supernatant and resuspend pellet in 100 µl A-LYS/IC mix by vortexing.
4. Incubate sample for 5 min at 95°C in a water bath. Briefly spin down.
5. Add 100 µl Neutralization Buffer (A-NB) and vortex sample for 5 sec.
6. Spin down for 5 min at full speed in a table top centrifuge and directly use 5 µl of the supernatant for PCR.
   In case the DNA solution is to be stored for an extended period of time, transfer supernatant to a new tube.

The extracted DNA may directly be used for downstream applications or can be stored at –20°C.

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 performance evaluation of the **GenoLyse®** kit was carried out with compatible test kits from Hain Lifescience, applying the conditions indicated in the respective instructions for use. The starting materials included in the respective instructions for use were tested. Until the present edition of the instructions on hand, the performance of the extraction method has not been validated with other test kits or other sample materials. Performance data can be requested through www.hain-lifescience.com
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.
This kit was not evaluated for DNA extraction from stool samples or blood as well as swab media containing inhibitors of PCR (e.g. alcohols, SDS). The kit was neither validated for extraction from fungi, parasites or viruses nor for extraction of RNA.

Troubleshooting

Problems in subsequent applications (e.g. amplification problems)
- DNA solution contains inhibitors. Ensure appropriate starting material.
- DNA solution contains protein contaminations. Include or extend centrifugation step of neutralized cell lysate and transfer supernatant to a new tube.
- Improper sampling, storage, transport, or preparation of specimen. Request 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 (see chapter Quality Control). Repeat extraction with new reagents.

Material Required but not Included in the Kit

- 0.9% sodium chloride solution (for protocol **B**)
- 1.5 ml screw cap tubes
- Adjustable pipettes for 20, 200, and 1000 µl
- Class II safety cabinet (for protocols **A** and **C**)
- Disposable gloves
- Disposable sterile pipette tips with filter
- Table top centrifuge, if applicable with aerosol-tight rotor
- Timer
- Vortexer
- Water bath, precision +/–1°C

Kit Contents

Order no.	**51612**	**51610**
Extractions	**12**	**96**
Lysis Buffer (A-LYS) contains <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
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Ordering Information

	Order no.
GenoLyse® (kit for manual DNA extraction of 12 samples)	51612
GenoLyse® (kit for manual DNA extraction of 96 samples)	51610

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).

Important Changes in IFU-51610-14

Chapter	**Change**
Intended Use, Procedure	The **GenoLyse**® kit can also be used with the new **GenoType CM*direct*** kit.

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