

GenoLyse[®]

VER 1.0

Instructions for Use

IFU-51610-08

CE

IVD

for in vitro diagnostic use only

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: **GenoType MTBDRplus**, **GenoType MTBDRsl**, **GenoQuick® CT**, and **GenoQuick® MTB**. Depending on the subsequent test, patient specimens and/or cultured material can be used as starting material.

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 genomic DNA may directly be used for downstream applications and can be stored at –20°C.

Storage and Disposal of Kit Constituents

Store all kit components at 2-8°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 material safety data sheets which can be downloaded from: www.hain-lifescience.com/products/msds.html

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.

Specimen Requirements

The applicable starting materials for the diagnostic test kit (**GenoType MTBDRplus**, **GenoType MTBDRsl**, **GenoQuick® CT**, or **GenoQuick® MTB**) 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.

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 risk patients (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.

Procedure

A. For use with the **GenoType MTBDRplus**, **GenoType MTBDRsl**, or **GenoQuick® MTB** assay:

1. When using patient specimens, transfer 500 µl of decontaminated sample material into a labeled 1.5 ml screw cap tube; when using bacteria grown in liquid media (only **GenoType MTBDRplus** and **GenoType MTBDRsl**), transfer 1 ml.
When using bacteria grown on solid medium (only **GenoType MTBDRplus** and **GenoType MTBDRsl**), 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 in a standard table top centrifuge with aerosol tight rotor.
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 with aerosol tight rotor 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.

B. For use with the **GenoQuick® CT** assay:

1. Rinse swab in transport medium or, in case a dry swab is used, 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.
2. Transfer 500 µl of sample material from 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 genomic DNA may directly be used for downstream applications and can be stored at –20°C.

Limitations

Strictly adhere to the established protocols and procedures in order to avoid contaminations and to obtain correct test results.

The performance evaluation of the **GenoLyse®** kit was carried out with compatible test kits from Hain Lifescience only, applying the conditions indicated in the respective instructions for use. 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.

Use of this kit is limited to qualified personnel well trained in the procedure and familiar with molecular biological methods.

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

Low DNA yield

- Insufficient cell lysis. Extend incubation time.
- Not enough cells in starting material. Ensure appropriate starting material and appropriate storage of starting material. Repeat DNA extraction where appropriate.

Degraded DNA

- Inappropriate storage of starting material.
- Aged starting material.

Request new specimens and repeat DNA extraction.

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.
- Use of too much DNA. Dilute DNA solution and repeat subsequent assay.

Material Required but not Provided

- 0.9% sodium chloride solution (for protocol B)
- 1.5 ml screw cap tubes
- Adjustable pipettes for 20, 200, and 1000 µl
- Disposable gloves
- Disposable sterile pipette tips with filter
- Table top centrifuge, where required with aerosol tight rotor
- Timer
- Vortex mixer
- Water bath, precision +/-1°C

Kit Contents

Order no. Extractions	51612 12	51610 96
Lysis Buffer (A-LYS) ready to use contains 1% nonionic tenside, <0.2% NaOH, dye	2 ml	12 ml
Neutralization Buffer (A-NB) ready to use contains buffer	2 ml	12 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 instrument biohazards and infectious disease transmitted by blood, body fluids, and tissue. 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-51610-08

Chapter	Change
	generally revised and restructured: <ul style="list-style-type: none"> – former chapter "Methodology" extended and split in new chapters "Intended Use" and "Principles of the Procedure" – former chapter "Storage and Precautions" extended and split in new chapters "Storage and Disposal of Kit Components", "Precautions for Handling Kit Components", and "Specimen Requirements" – new chapters: "Ordering Information", "References", "Important Changes"
Intended Use, Procedure	The GenoLyse® kit was approved for use with the GenoType MTBDRsl . From now on the GenoLyse® kit can be processed with culture samples for subsequent use with the GenoType MTBDRplus or with the GenoType MTBDRsl .
Troubleshooting	explanation of steps to be taken
Ordering Information	GenoLyse® is now also available as kit for 12 extractions.



51610-08-02



Hain Lifescience GmbH

Hardwiesenstraße 1, 72147 Nehren, Germany
www.hain-lifescience.de, +49 (0) 74 73- 94 51- 0