

Manual

MutaCLEAN® PLUS

Reagent for the enzymatic release of DNA from bacterial cultures and swabs

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1 INTENDED USE

The MutaCLEAN[®] PLUS Nucleic Acid Release Reagent is designed for the release of DNA from bacterial cultures and swabs. The crude lysates can be directly applied in molecular assays, e.g. real time PCR.

2 PRINCIPLE OF THE TEST

The MutaCLEAN[®] PLUS Nucleic Acid Release Reagent causes the lysis of bacteria. The lysis is performed for 15 minutes at room temperature.

Nucleic acids released with MutaCLEAN[®] PLUS Nucleic Acid Release Reagent can be analysed by employing the supernatants obtained directly in the subsequent molecular assay.

Pooling of the lysates prior to analysis is possible; however, it is subject to the purpose and regulations of the particular application.

3 PACKAGE CONTENTS

 $4\,x\,8.75\,ml$ MutaCLEAN* PLUS Nucleic Acid Release Reagent, sufficient for 100 reactions.

4 EQUIPMENT AND REAGENTS TO BE SUPPLIED BY USER

- · Laboratory equipment according to national safety instructions
- Sterile pipet tips with filters
- Nuclease-free 1.5 or 2.0 ml microcentrifuge tube
- Optional: Liquid handling system for automation

5 TRANSPORT, STORAGE AND STABILITY

The MutaCLEAN[®] PLUS Nucleic Acid Release Reagent is shipped on dry ice. It must be stored at \leq 20 °C. If properly stored, it is stable until the date of expiry printed on the label.

Please note that improper storage will adversely impact nucleic acid purification due to decreased enzymatic activity.

6 GENERAL INFORMATION

6.1 Important notes

- The MutaCLEAN® PLUS Nucleic Acid Release Reagent must be utilised by qualified personnel only.
- Good Laboratory Practice (GLP) has to be applied.
- Clinical samples must always be regarded as potentially infectious material and all equipment used has to be treated as potentially contaminated.

6.2 General precautions

- Avoid contact of the buffer with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with large amount of water. Burns can occur if left untreated. If the reagent spills, dilute with water before wiping dry.
- Never store or use the buffer near human or animal food.
- Always wear gloves and follow standard safety precautions when handling these buffers.

6.3 Handling requirements

- Exercise the normal precautions required for handling all laboratory reagents.
- Do not pool reagents from different lots or from different bottles of the same lot. Immediately after usage, close all bottles in order to avoid leakage, varying buffer concentrations or buffer conditions. After first opening, store all bottles in an upright position.
- Do not use a kit after its expiration date.

6.4 Laboratory procedures

- All sourced material and all resulting waste should be considered potentially infectious. Thoroughly clean and disinfect all work surfaces with disinfectants recommended by the local authorities.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection when handling specimens and kit reagents.

- Avoid microbial and nuclease contamination of reagents when removing aliquots from reagent bottles.
- The use of sterile disposable pipettes is recommended.
- Wash hands thoroughly after handling samples and test reagents.

6.5 Waste handling

- Dispose of unused reagents and waste should occur in accordance with country, federal state and local regulations.
- Material Safety Data Sheets (MSDS) are available upon request.

7 SAMPLE MATERIAL

Starting material are respiratory swabs (e.g. buccal swabs, nasal swabs, etc.) or bacterial culture samples.

8 PROCEDURE

- Pipet 350 µl MutaCLEAN[®] PLUS Nucleic Acid Release Reagent into an appropriate tube (e.g. 2 ml reaction tube, safe lock).
- Place the swab tip or the picked bacterial colony into the reaction tube and break or cut off the applicator at a length that allows the tube to be closed.
- Close reaction tube tightly.
- Vortex thoroughly 4–5 times
- Incubate for 15 min at room temperature.

9 STORAGE OF CRUDE LYSATES

For storage conditions of inactivated crude MutaCLEAN® PLUS Nucleic Acid Release Reagent lysates, please refer to table 1.

able 1:	Storage conditions for crude lysates
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Time	Storage Condition
up to 6 hours	Room temperature
up to 24 hours	+2 to +8 °C
long term storage	≤ -18 °C

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10 TROUBLESHOOTING

The following troubleshooting guide is included to help you with possible problems that may arise in a subsequent PCR.

Neither sample nor Internal Control show a PCR signal

Concentration of PCR inhibitors in the sample too high.

Components present in the sample may inhibit the PCR. Therefore, dilute the supernatant 1:10 in dH_2O (PCR grade). If necessary, extract the nucleic acid from the crude lysate with a commercial extraction kit (e.g. MutaCLEAN[®] Universal RNA/DNA) and repeat PCR analysis.

Taq-Polymerase damaged by MutaCLEAN® PLUS

PCR run should be started immediately (max. within 15 – 20 min.) after pipetting the MutaCLEAN[®] PLUS lysate into the reaction mix.

Negative PCR result for a known-positive sample, Internal Control shows no inhibition

Kit stored under non-optimal conditions or kit expired

Store kit at \leq -20°C. Do not use after the date of expiry printed on the label.

Incorrect incubation conditions

Make sure incubation conditions comply with the protocol.

11 ABBREVIATIONS AND SYMBOLS

DNA	Deoxyribonucleid Acid	→REF	To be used with
PCR	Polymerase Chain Reaction	REF	Catalog number
	Manufacturer	Σ	Contains sufficient for <n> test</n>
CONTENT	Content	X	Upper limit of temperature
i	Consult instruc- tions for use		Use by
		LOT	Lot number

12 LITERATURE

1. Sambrook, J. and Russell, D.W.: Molecular Cloning, 2001.

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