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Manual

MutaCLEAN® PLUS

Reagent for the enzymatic release of nucleic acid from swabs and cell culture suspensions

Valid from 2017-04-28

REF **KG1036**

Σ 100

-20 °C

IVD

CE

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1 INTENDED USE

The MutaCLEAN® PLUS lysis buffer is designed for the release of nucleic acids from swabs and cell culture suspensions. The crude lysates can be directly applied in (real time) PCR or (real time) RT-PCR.

2 PRINCIPLE OF THE TEST

The MutaCLEAN® PLUS lysis buffer causes the digestion of mammalian cells, gram-positive and gramnegative bacteria, and virus particles. The digestion is performed for 15 minutes at room temperature.

Nucleic acids released with MutaCLEAN® PLUS lysis buffer 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 lysis buffer, 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: Block incubator or laboratory furnace
- Optional: Liquid handling system for automation
- Optional: BLP-DNA (bacterium-like particles, KG7013, see chapter 10)

5 TRANSPORT, STORAGE AND STABILITY

The MutaCLEAN® PLUS lysis buffer is shipped on dry ice. It must be stored at ≤ 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 extraction 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 swabs (e. g. buccal swabs, nasal swabs, etc.) or cell culture suspensions.

Table 1: Volumes of MutaCLEAN® PLUS lysis buffer and pre-treatment of the sample for different sample matrices.

Sample material	Volume/ Amount	Volume of MutaCLEAN® PLUS lysis buffer	Pre-treatment of the sample
Swab (buccal, nasal, etc.)	1 swab	350 µl	-
Cell culture suspension	up to 1×10^{11} cells	350 µl	Pellet cells by centrifugation and discard supernatant.

8 PROCEDURE

8.1 Release of nucleic acids from swabs

- Pipet 350 µl MutaCLEAN® PLUS lysis buffer into an appropriate tube (e. g. 2 ml reaction tube, safe lock).
- Place the swab tip 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. Optionally, this incubation step can be performed at 37 °C.
- Inactivate proteolytic enzymes according to chapter 8.3, Inactivation of MutaCLEAN® PLUS lysis buffer.

8.2 Release of nucleic acids from cell cultures

- Pellet up to 1 x 10^{11} cells by centrifugation and discard supernatant.
- Resuspend pellet in 350 µl MutaCLEAN® PLUS lysis buffer by vigorous vortexing.
- Incubate for 15 min at room temperature. Optionally, this incubation step can be performed at 37 °C.
- Inactivate proteolytic enzymes according to chapter 8.3, Inactivation of MutaCLEAN® PLUS lysis buffer.

8.3 Inactivation of MutaCLEAN® PLUS lysis buffer

If the molecular assay requires enzymatic activity, e. g. reverse transcription of RNA, the MutaCLEAN® PLUS lysis buffer components have to be inactivated by heat. If a PCR is to be performed, heat inactivation of MutaCLEAN® PLUS lysis buffer can be achieved by elongating the intial denaturation step of the PCR to 15 min. For analysis of the lysate in a subsequent reverse transcription PCR, follow the steps mentioned below:

- After digestion, incubate the sample for 10 min at 97 °C. For sufficient denaturation of the proteolytic enzymes, compliancy to this protocol is absolutely essential!
- Let the sample cool down for 10 min.
- Briefly centrifuge heat inactivated samples in order to collect condensed water from the tube lid.
- Take aliquots close to the surface of the supernatant in order to avoid cell debris being transferred to the following analysis (e. g. PCR). Do not shake!

9 Storage of crude lysates

For storage conditions of inactivated crude MutaCLEAN® PLUS lysis buffer lysates, please refer to table 2.

Table 2: Storage conditions for inactivated crude lysates

Time	Storage Condition
up to 6 hours	Room temperature
up to 24 hours	+2 to +8 °C
long term storage	≤ -18 °C

10 ASSAY VALIDATION

Extraction control

Use VLP-RNA (available upon request), VLP-DNA (available upon request) or BLP-DNA (KG7013) as an extraction control. E. g. add 5 µl of the extraction control per reaction directly to MutaCLEAN® PLUS lysis buffer and co-extract with the nucleic acid of the sample. The Ct value of the extraction control in the subsequent real time (RT-) PCR needs to meet the validation criteria of the respective real time (RT-) PCR Kit.

11 TROUBLESHOOTING

The following troubleshooting guide is included to help you with possible problems that may arise when isolating nucleic acid from different types of sample material or in a subsequent PCR.

Neither sample nor Internal Control show a PCR signal

The inactivation of the proteolytic enzymes in MutaCLEAN® PLUS lysis buffer was not effective.

Heat the sample once more to 97 °C for 10 min. Repeat PCR analysis.

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 dH2O (PCR grade). If necessary, extract the nucleic acid from the crude lysate with a commercial extraction kit (e. g. MutaCLEAN® Universal RNA/DNA, KG1038)) and repeat PCR analysis.

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

Incorrect storage conditions for one or more components or kit expired

Check the storage conditions and the date of expiry printed on the kit label. If necessary, use a new kit and make sure kit components are stored as described in chapter "Transport, Storage and Stability".

Incorrect incubation conditions

Make sure incubation conditions comply with the protocol.

12 ABBREVIATIONS AND SYMBOLS

DNA	Deoxyribonucleid Acid	BLP	Bacterium-like particles
CT	Cycle threshold	VLP	Virus-like particles
PCR	Polymerase Chain Reaction	→REF	To be used with
RT	Reverse Transcriptase	REF	Catalog number
	Manufacturer		Contains sufficient for <n> test
CONT	Content		Upper limit of temperature
	Consult instructions for use		Use by
		LOT	Lot number

13 LITERATURE

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