Instruction for Use

NukEx

Nucleic Acid Release Reagent

For general laboratory use.
For *in vitro* use only.

Reagent for the enzymatic release of nucleic acid from tissue samples, ticks, insects and swabs.

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1 Intended Use

The NukEx Nucleic Acid Release Reagent is designed for the release of nucleic acids from tissue samples (e.g. cartilage tissue, skin biopsies, and adenoid tissue), ticks, insects and swabs. The crude lysates can be directly applied in (real time) PCR or (real time) RT-PCR.

2 Mode of Action

For the analysis of nucleic acids by polymerase chain reaction (PCR) or RT-PCR, the isolation of the analyte from various sample materials is required.
The NukEx Nucleic Acid Release Reagent allows for the enzymatic digestion of tissue samples, ticks, insects and swabs. The digestion can be performed either up to one hour at 60 °C or over night at room temperature. Afterwards, the proteolytic enzyms have to be inactivated by heat.
Nucleic acids released with NukEx Nucleic Acid Release Reagent can be analysed by employing the crude lysates directly in the subsequent molecular assay.
Depending on the assay to be performed, additional extraction of the nucleic acid with a commercially available extraction kit is advisable. Pooling of the lysates prior to analysis is possible; however, it is subject to the purpose and regulations of the particular application.

3 Components

3x 10 ml NukEx Nucleic Acid Release Reagent sufficient for appr. 100 reactions.

4 Equipment and Reagents to be Supplied by User

- Laboratory equipment according to national safety instructions.
- Sterile pipet tips with filter
- Nuclease-free 1.5 or 2.0 ml microcentrifuge tube
- Optional: Blockincubator or laboratory furnace
- Optional: Liquid handling systems for automation

5 Transport and Storage

NukEx Nucleic Acid Release Reagent is shipped on dry ice or at ambient temperature. NukEx Nucleic Acid Release Reagent must be stored ≤ -18°C immediately after receipt. If properly stored, the product is stable until the date of expiry printed on the label.

6 General Information

6.1 Important Notes

- The NukEx 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

- Do not let buffers touch your skin, eyes, or mucous membranes. If contact does occur, wash the affected area immediately with large amounts of water; otherwise, the reagent may cause burns. If you spill the reagent, dilute the spill with water before wiping it up.
- Never store or use the buffers 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.
- Avoid contact of the kit components 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.
- Use only calibrated pipettes.

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 from gerbion.

7 Sample Material

Tissue samples like cartilage tissue, skin biopsies or adenoid tissue, ticks and insects.

Table 1: Volumes NukEx Nucleic Acid Release Reagent and pre-treatment of the sample for different sample matrices.

Sample material	Volume/ Amount	Volume of NukEx Nucleic Acid Release Reagent	Pre-treatment of the sample
Tissue samples (e.g. ear notches)	≤ 30 mg	Ratio 1:10 sample weight to NukEx Nucleic Acid Release Reagent volume (e.g. 30 mg sample + 300 µl NukEx Nucleic Acid Release Reagent	-
Ticks/insects	≤ 30 mg	300 µl	Homogenization of tissue in NukEx Nucleic Acid Release Reagent e.g. with NukEx TS (Cat. No. G06007)
swab (buccal, nasal, etc.)	1 swab	300 µl	-

8 Procedure

Procedures below are for preparing nucleic acids from approx. 30 mg tissue sample. If other sample sizes or other sample matrices are to be used please refer to Table 1 for appropriate buffer volumes.

8.1 Tissue Digestion

- Transfer sample into an appropriate tube (e.g. 2 ml reaction tube, safe lock).
- Add 300 µl NukEx Nucleic Acid Release Reagent to 30 mg tissue sample (or in a ratio 1:10 sample weight to reagent volume; e.g. 30 mg sample + 300 µl NukEx Nucleic Acid Release Reagent).
- Ensure that samples are fully immersed in NukEx Nucleic Acid Release Reagent.
- Lock reaction tube well.
- Incubate for 30 min. to 1 h at 60 ± 2°C or over night at room temperature.
- Inactivate proteolytic enzymes according to 8.3 Inactivation of NukEx Nucleic Acid Release Reagent.

8.2 Release of Nucleic Acids from Swabs

- Pipet 300 µl NukEx Nucleic Acid Release Reagent 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 thouroughly 4- 5 times
- Incubate for 15 min to 30 min at 60 ± 2°C.
- Inactivate proteolytic enzymes according to 8.3 Inactivation of NukEx Nucleic Acid Release Reagent.

8.3 Inactivation of NukEx Nucleic Acid Release Reagent

Prior to using the samples in molecular assays, the proteolytic enzymes must be inactivated by heat:

- After tissue digestion incubate the sample for min. 10 min at 97± 2°C. For sufficient denaturation of the proteolytic enzymes compliancy to this protocol is absolutely essential!
- Let the sample cool down for min. 5 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!
- Crude heat inactivated lysates can be employed directly in various molecular assays.

9 Storage of Crude Lysates

For storage conditions of inactivated crude NukEx Nucleic Acid Release Reagent 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

Optionally, use VLP-RNA (G07008), VLP-DNA (G07012) or BLP-DNA (G07013) as an extraction control. E.g. add 5 µl of the Extraction Control per reaction directly to the NukEx Nucleic Acid Release Reagent 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 in a subsequent PCR. For further questions concerning nucleic acid isolation, please do not hesitate to contact our scientists on info@gerbion.com.

Neither sample nor Internal Control show a PCR signal	
The inactivation of the proteolytic enzymes in NukEx Nucleic Acid Release Reagent was not effective.	Heat the sample once more to 97 ± 2°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 dH_2O (PCR grade). If necessary, extract the nucleic acid from the crude lysate with a commercial extraction kit (e.g. NukEx Pure RNA/DNA, Cat. No. G05004) and repeat PCR analysis.
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 ≤ -18°C. Do not use after the date of expiry printed on the label.
Incorrect incubation conditions	Make sure incubation conditions comply with the protocol (page 6)

12 Abbreviations and Symbols

DNA	Desoxyribonucleic Acid	REF	Catalog number
RNA	Ribonucleic Acid		Contains sufficient for <n> test
PCR	Polymerase Chain Reaction		Upper limit of temperature
RT	Reverse Transcriptase		Manufacturer
LOT	Batch code		Use by YYYY-MM-DD
CONT	Content		Consult instructions for use

13 Literature

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