Instruction for Use

NukEx Extreme SC
NukEx Extreme SL

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

For isolation of nucleic acids using bead beating.

REF	G05022-100	SC – Bead Beating Tube Screw Cap
	G05023-100	SL – Bead Beating Tube Safe Lock
Σ	100	
+25°C +18°C		European patent pending. (EP 18 000 803.9)

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1 Intended Use

The NukEx Extreme SC and NukEx Extreme SL Kits are designed for the rapid purification of nucleic acids from a wide range of difficult samples (e.g. human samples, veterinary samples, insects, food samples, etc.).
The kits are designed for use with tissue mills (e.g. Fast Prep cell disruptor - NukEx Extreme SC or TissueLyser - NukEx Extreme SL) for mechanical disruption of samples and subsequent extraction of nucleic acids using NukEx spin columns. The purified nucleic acids RNA and DNA can be used directly as template for real time (Reverse Transcription-) PCR or any kind of enzymatic reactions.

2 Mode of Action

For the analysis of nucleic acids by real time (Reverse Transcription-) PCR, the isolation of the analyte from various sample materials is required. To this end, the sample is lysed by mechanical disruption in NukEx Bead tubes in the presence of a proprietary extraction buffer system.
After binding of nucleic acids to the surface of the spin columns, the columns are washed to remove contaminants and salts using Inhibitor Removal Buffer and Wash Buffer. Residual ethanol from previous wash steps is removed by centrifugation at maximum speed. Finally, highly pure RNA / DNA is eluted with low-salt Elution Buffer. Purified RNA / DNA can directly be used for downstream applications.

1. Samples are lysed by mechanical disruption in the presence of Extraction Buffer 1 (EX1) and subsequent adding of Extraction Buffer 2 (EX2).
2. Nucleic acids are bound to the surface of the spin columns.
3. Bound nucleic acids are washed with Inhibitor Removal Buffer (P2) in order to remove PCR inhibitors from the sample.
4. Bound nucleic acids are washed with Wash Buffer (P3) in order to purify them from salts, proteins and other cellular impurities.
5. Purified nucleic acids are eluted from the NukEx Spin Columns with the Elution Buffer (P4).

3 Components

NukEx Extreme G05022-100 / G05023-100 are designed for 100 isolations.

Table 1: Components of the NukEx Extreme isolation kit

Labelling		Content
EX1	Extraction Buffer 1 (EX1)	1 x 60 ml
EX2	Extraction Buffer 2 (EX2)	1 x 60 ml
P2	Inhibitor Removal Buffer (P2)	1 x 33 ml add 20 ml absolute ethanol
P3	Wash Buffer (P3)	1 x 20 ml add 80 ml absolute ethanol
P4	Elution Buffer (P4)	1 x 5 ml
	NukEx Spin Columns	2 x 50 pieces
NEBC	NukEx Bead SC (incl. in G05022-100)	1x 100 vials
NEBL	NukEx Bead SL (incl. in G05023-100)	1x 100 vials

All solutions are clear and should not be used when precipitates have formed. Warm the solutions at +18 to +25°C or in a 37°C water bath until the precipitates have dissolved.

4 Equipment and Reagents to be Supplied by User

For use of NukEx Extreme SC the use of FastPrep- or Precellys homogenizers is recommended. For use of NukEx Extreme SL the use of TissueLyser homogenizer is recommended. The kits can also be used with other common homogenizers.

- Homogenizer (e.g. FastPrep® Cell Disruptor, Precellys, TissueLyser, or equivalent)
- distilled water (sterile Aqua dest.)
- Sterile pipet tips with filter
- 250 ml screw cap vial (e.g. Sarstedt Art. No. 75.9922.534)
- Nuclease-free Collection Tubes (gerbion, Cat. G06008)
- Nuclease-free 1.5 ml or 2.0 ml microcentrifuge tube
- Tabletop microcentrifuge capable of 13,000 x g centrifugal force
- Absolute ethanol
- Thermoblock or laboratory furnace
- Laboratory equipment according to national safety instructions.

5 Transport, Storage and Stability

The NukEx Extreme Kit components must be stored at +18 to +25°C. If properly stored, all kit components are stable until the date of expiry printed on the label. Please note, that improper storage at +2 to +8°C (refrigerator) or ≤-18°C (freezer) will adversely impact nucleic acid purification when precipitates form in the solutions. Therefore, NukEx Extreme Kits are always shipped at +18 to +25°C.

6 General Information

6.1 Important Notes

- The NukEx Extreme Kits 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 Precautions

- NukEx Extreme Inhibitor Removal Buffer contains guanidine hydrochloride which is an irritant. Always wear gloves and follow standard safety precautions to minimize contact when handling. Do not use bleach for waste treatment!
- Do not let these 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 Inhibitor Removal 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.
- Do not use any modified ethanol.
- 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 Preparation of Solutions

Table 2: Preparation of NukEx Extreme Solutions.

Label	Reconstitution/ Preparation G05022-100/ G05023-100	Storage and Stability	Purpose
Inhibitor Removal Buffer (P2)	Add 20 ml absolute ethanol to Inhibitor Removal Buffer and mix well. Label and date bottle accordingly after adding absolute ethanol.	Store at +18 to +25°C. Stable through the date of expiry printed on the kit label.	Removal of PCR inhibitors from the bound nucleic acid.
Wash Buffer (P3)	Add 80 ml absolute ethanol to Wash Buffer and mix well. Label and date bottle accordingly after adding absolute ethanol.	Store at +18 to +25°C. Stable through the date of expiry printed on the kit label.	Removal of salts, proteins and other residual impurities from the bound nucleic acid.

8 Sample Material

Purification of nucleic acids from a wide range of difficult sample material, such as the following:

- Human samples (tissue, stool)
- Veterinary samples (tissue, feces)
- Insects and ticks
- Food samples
- Environmental samples
- Plant material

Table 3: Pre-treatment for different sample matrices.

Sample material	Volume/ Amount	Pre-treatment of the sample
stool, feces	pea-size up to 1 g for MAP extraction see 10.1.1.	Prepare a suspension in 2.5 ml sterile Aqua dest., vortex and briefly spin down sediments at low speed (1,500 x g) for 2 min. Transfer 1.5 ml of the supernatant into a 2 ml reaction tube. Centrifuge at 8,000 x g. Discard supernatant and add 600 µl of Extraction Buffer 1(EX 1).
animal/ human tissues	up to 0.5 g	Transfer tissue to a NukEx Bead tube (NEBC or NEBL) and add 600 µl of Extraction Buffer 1(EX 1).
plant tissues	up to 0.5 g	Transfer tissue to a NukEx Bead tube (NEBC or NEBL) and add 600 µl of Extraction Buffer 1(EX 1).
animal / human cells	up to 10^8	Harvest up to 2 x 10^8 cells. Transfer the cell suspension to a NukEx Bead tube (NEBC or NEBL). Spin down cells at low speed (3,000 x g) for 2 min. Discard supernatant and add 600 µl of Extraction Buffer 1(EX 1).

9 Preparation Time

The preparation time needed is always dependent on the number of samples to be prepared.

Table 4: Preparation times for the isolation of nucleic acids with NukEx Extreme Kit.

	DNA/ RNA
Total time	approx. 20 minutes
Hands-on time	less than 5 minutes

10 Procedure

Long term storage of extracted nucleic acids is recommended at ≤-65 °C.

10.1 Isolation of nucleic acids

Important information:

Depending on the extraction control used, the Control-DNA/-RNA or Internal Process Control (IPC) can be added to the samples before or after homogenization/mechanical disruption. The user needs to check, if the Control-DNA/-RNA or IPC used are stable enough to resist the bead-beating process. If the Control-DNA/-RNA or IPC must be added after bead-beating, it must be tested, if a working solution of EX2 and Control-DNA/-RNA or IPC can be made and if the Control-DNA/-RNA or IPC are stable in this working solution. If this is not the case, Control-DNA/-RNA or IPC must be added to the homogenate after pipetting EX2 (see below).

10.1.1 Isolation of nucleic acids from bovine, ovine and caprine fecal samples

Feces quantity can vary from 1 g to 10 g of feces (=X). Dilute 1 quantity of fecal sample in 2.5 volumes of sterile Aqua dest. (dilution (w/v) / 2.5); e.g. 3 g with 7.5 ml sterile Aqua dest. or 5 g with 12.5 ml sterile Aqua dest. or 10 g with 25 ml sterile Aqua dest. Respect the weight/volume ratio. The sensitivity and reproducibility can be improved when the quantity of fecal sample is higher.
Environmental samples (e.g. sock samples) are treated as a fecal sample. Disolve 3 g – 5 g of the sample in sterile Aqua dest. (see page 10).

Procedure for feces

- Add 2.5 ml (or x-fold) sterile Aqua dest. to 1 g +/- 0.2 g (or x-fold) of feces.
- Vortex thoroughly for approx. 30 sec.
- Centrifuge for 2 min at 1,500 x g or let the sample settle for app. 10 min.
- Transfer 1.5 ml of the supernatant into a NukEx Bead tube (NEBC or NEBL).
- Centrifuge for 5 min at 8,000 x g.
- Discard supernatant.
- Add 600 µl Extraction Buffer 1 (EX1), close vial tightly.
- Homogenize in a FastPrep® Cell Disruptor 3x 40 sec at level 6.5 with 1 min pause in between. Equivalent instruments such as TissueLyser (Qiagen, 3x 4 min at 30 Hz) or Precellys (Bertin Technologies, 3x 30 sec at 6,800 rpm) can also be used.

- Before opening the vial, let the foam settle for 1-2 min or centrifuge briefly to avoid cross-contaminations.
- Add 600 µl Extraction Buffer 2 (EX2) and mix briefly.
- Centrifuge for 5 min at 3,000 x g.
- Use 800 µl of the supernatant for isolation of nucleic acids with NukEx Spin Columns (shown in 10.2.).

Procedure for sock samples

- Place sock sample in a 250 ml screw cap vial (e.g. Sarstedt Art. No. 75.9922.534), vial can also be used for transport purposes.
- Add 100 ml sterile Aqua dest.
- Shake vial vigorously for app. 20 sec.
- Transfer 10 ml of the supernatant into a 15 ml or 50 ml reaction tube.
- Centrifuge for 5 min at 4,500 x g.
- Discard supernatant.
- Resuspend the pellet in 2.0 ml sterile Aqua dest.
- Transfer 1.5 ml of the suspension into a NukEx Bead tube (NEBC or NEBL).
- Centrifuge for 5 min at 8,000 x g.
- Discard supernatant.
- Add 600 µl Extraction Buffer 1 (EX1), close vial tightly, vortex vigorously.
- Homogenize in a FastPrep® Cell Disruptor 3 x 40 sec at level 6.5 with 1 min pause in between. Equivalent instruments such as TissueLyser (Qiagen, 3x 4 min at 30 Hz) or Precellys (Bertin Technologies, 3x 30 sec at 6,800 rpm) can also be used.
- Before opening the vial, let the foam settle for 1-2 min or centrifuge briefly to avoid cross-contaminations.
- Add 600 µl Extraction Buffer 2 (EX2) and mix briefly.
- Centrifuge for 5 min at 3,000 x g.
- Use 800 µl of the supernatant for isolation of nucleic acids with NukEx Spin Columns (shown in 10.2.).

10.1.2 Isolation of RNA and DNA from solid materials

Procedure

- Transfer tissue (up to 0.5 g) to a NukEx Bead tube (NEBC or NEBL).
- Add 600 µl Extraction Buffer 1 (EX1).
- Homogenize in a FastPrep® Cell Disruptor 3 x 40 sec at level 6.5 with 1 min pause in between. Equivalent instruments such as TissueLyser (Qiagen, 3x 4 min at 30 Hz) or Precellys (Bertin Technologies, 3x 30 sec at 6,800 rpm) can also be used.
- Before opening the vial, let the foam settle for 1-2 min or centrifuge briefly to avoid cross-contaminations.
- Add 600 µl Extraction Buffer 2 (EX2) and mix briefly.
- Centrifuge for 5 min at 3,000 x g.
- Use 800 µl of the supernatant for isolation of nucleic acids NukEx Spin Columns (shown in 10.2.).

10.1.3 Procedure for liquid culture (bacterial culture, cell culture) or culture suspensions

For cuts: purge colonies from the agar surface using 1 ml sterile Aqua dest.
For colony-suspensions: pick colonies from the agar surface and re-suspend in 1 ml sterile Aqua dest.
For liquid cultures: use 1 ml of the suspended culture material

- Transfer 1 ml of the suspension to a NukEx Bead tube (NEBC or NEBL).
- Centrifuge for 5 min at 3,000 x g.
- Discard supernatant.
- Add 600 µl Extraction Buffer 1 (EX1).
- Homogenize in a FastPrep® Cell Disruptor 3 x 40 sec at level 6.5 with 1 min pause in between. Equivalent instruments such as TissueLyser (Qiagen, 3x 4 min at 30 Hz) or Precellys (Bertin Technologies, 3x 30 sec at 6,800 rpm) can also be used.
- Before opening the vial, let the foam settle for 1-2 min or centrifuge briefly to avoid cross-contaminations.
- Add 600 µl Extraction Buffer 2 (EX2) and mix briefly.
- Centrifuge for 5 min at 3,000 x g.
- Use 800 µl of the supernatant for isolation of nucleic acids with NukEx Spin Columns (shown in 10.2.).

10.2 Detailed Protocol for Spin Column Extraction

Step 1

- Transfer 800 µl of the lysate (supernatant) into the reservoir of the NukEx Spin Column

Step 2

- Centrifuge 1 min at 8,000 × g.
- Remove the NukEx Spin Column from the Collection Tube, discard the flowthrough liquid and the Collection Tube.
- Replace the Collection Tube.

Step3

- Add **500 µl Inhibitor Removal Buffer (P2)** into the reservoir of the NukEx Spin Column.
- Centrifuge 30 s at 8,000 × g.
- Remove the NukEx Spin Column from the Collection Tube, discard the flowthrough liquid and the Collection Tube.
- Replace the Collection Tube.

Step 4

- Add **450 µl Wash Buffer (P3)** into the reservoir of the NukEx Spin Column.
- Centrifuge 30 s at 8,000 × g.
- Remove the NukEx Spin Column from the Collection Tube, discard the flowthrough liquid and the Collection Tube.
- Replace the Collection Tube.

Step 5

- Add **450 µl Wash Buffer (P3)** into the reservoir of the NukEx Spin Column.
- Centrifuge 30 s at 8,000 × g.
- Centrifuge 10 s at maximum speed (13,000 × g) in order to completely remove the ethanol from the column

Step 5

- Transfer the NukEx Spin Column into a nuclease-free 1.5 ml microcentrifuge tube.
- Add **50 µl Elution Buffer (P4)** into the reservoir of the NukEx Spin Column.
- Incubate for 1 min at room temperature.
- Centrifuge 1 min at 8,000 × g.
- The eluate contains purified nucleic acid.

Important Note: Withdrawal of the Collection Tubes after each centrifugation step is highly recommended in order to avoid cross-contaminations.

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. Especially when working with complex sample matrices such as fatty tissue, whole blood or highly contaminated environmental samples, preparation of samples can be crucial. For protocols on sample materials not covered in this manual or for further questions concerning nucleic acid isolation, please do not hesitate to contact our scientists on info@gerbion.com.

Low nucleic acid yield	
Carry-over of ethanol from wash buffers	Be sure to remove all of the ethanolic wash solution from the final wash, as residual ethanol interferes with downstream applications.
Ethanol evaporation from wash buffers	Close buffer bottles tightly, avoid ethanol evaporation from buffer bottles as well as from buffer filled in reservoirs. Do not reuse buffers from buffer reservoirs.
Kit stored under non-optimal conditions.	Store kit at +18 to +25°C at all times upon arrival.
Buffers or other reagents were exposed to conditions that reduced their effectiveness	Store all buffers at +18 to +25°C. Close all reagent bottles tightly after each use to preserve pH and stability and to prevent contamination.
Ethanol not added to Inhibitor Removal Buffer (P2) and/or Wash Buffer (P3)	Add absolute ethanol to the buffers before using. After adding ethanol, mix the buffers well and store at +18 to +25°C. Always mark the buffer vials to indicate whether ethanol has been added or not.
Glass fibers, which might co-elute with nucleic acid, scatter light	Spin down eluate for 1 min at max. speed and transfer supernatant into a new sterile 1.5 ml microcentrifuge tube.

12 Abbreviations and Symbols

DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
PCR	Polymerase Chain Reaction
RT	Room Temperature

EXTRACTION BUFFER 1 EX1	Extraction Buffer 1 (EX1)
EXTRACTION BUFFER 2 EX2	Extraction Buffer 2 (EX2)
IR BUFFER P2	Inhibitor Removal Buffer (P2)
WASH BUFFER P3	Wash Buffer (P3)
ELUTION BUFFER P4	Elution Buffer (P4)
SPIN COLUMNS	Spin Columns
NUKEX BEAD SC NEBC	NukEx Bead SC (NEBC)
NUKEX BEAD SL NEBL	NukEx Bead SL (NEBL)

REF	Catalog number
	Contains sufficient for <n> test
	Upper limit of temperature
	Manufacturer
	Use by YYYY-MM-DD
LOT	Batch code
CONT	Content
	Consult instructions for use

13 Literature

[1] James H. Jorgensen, Michael A. Pfaller, Karen C. Carroll. Manual of Clinical Microbiology, 11th Edition, 2015.

[2] Richard L. Hodinka, Benjamin Pinsky. Clinical Virology Manual, 5th Edition, 2016.