Instruction for Use

NukEx Extreme SC
NukEx Extreme SL

For extraction of nucleic acids.

REF	G05022-100	SC – Bead Beating Tube Screw Cap
	G05023-100	SL – Bead Beating Tube Safe Lock
Σ	100	

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1 Intended Use

The NukEx Extreme SC and NukEx Extreme SL Kits are designed for the rapid extraction 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 homogenizers for mechanical disruption of samples and subsequent purification of nucleic acids using spin columns. The purified nucleic acids can be used directly as template for real time (Reverse Transcription-) PCR or any kind of enzymatic reactions.

2 Mode of Action

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

3 Components

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

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

- Homogenizer (e.g. FastPrep, Precellys, TissueLyser or equivalent)
- 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 Mag Extreme Kits are shipped at ambient temperature. Kits 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.

6 General Information

- 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.
- NukEx Extreme Inhibitor Removal Buffer contains guanidine hydrochloride which is an irritant. Always wear gloves and follow standard safety precautions.
- 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.
- 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.
- Do not use any modified ethanol.

7 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.

8 Preparation of Solutions

Table 2: Preparation of NukEx Extreme Solutions.

Label	Preparation G05022-100/ G05023-100	Storage and Stability
Inhibitor Removal Buffer (P2)	Add 20 ml absolute ethanol to Inhibitor Removal Buffer and mix well. Label and date bottle accordingly.	Store at +18 to +25°C. Stable through the date of expiry printed on the kit label.
Wash Buffer (P3)	Add 80 ml absolute ethanol to Wash Buffer and mix well. Label and date bottle accordingly	Store at +18 to +25°C. Stable through the date of expiry printed on the kit label.

9 Sample Material

- 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 extraction from bovine, ovine, caprine feces, esp. M. paratuberculosis DNA	see 10.1.
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).

10 Extraction 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 Extraction from bovine, ovine, 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., 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.

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 10.3).

Procedure for sock samples

- Place sock sample in a 250 ml screw cap vial.
- 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 by pipetting cautiously.
- Add **600 µl Extraction Buffer 1 (EX1)**, close vial tightly.
- 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 10.3).

10.2 Nucleic Acid Extraction from solid materials

- 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 10.3.).

10.3 Protocol for Spin Column Extraction

Step 1

- Transfer **800 µl of the lysate** (supernatant) into 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 sec 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 sec 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 sec at 8,000 × g.
- Centrifuge 10 s at maximum speed (13,000 × g) in order to completely remove the ethanol from the column

Step 6

- 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

For protocols on sample materials not covered by this manual or for further questions concerning nucleic acid extraction, please do not hesitate to contact our scientists on info@gerbion.com.

Troubleshooting	
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.

12 Abbreviations and Symbols

DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
PCR	Polymerase Chain Reaction
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
Σ	Contains sufficient for <n> test
+25°C +18°C	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.