Instruction for Use

NukEx Mag Extreme SC
NukEx Mag Extreme SL

For extraction of nucleic acids.

REF	G05024-100	SC – Bead Beating Tube Screw Cap
	G05025-100	SL – Bead Beating Tube Safe Lock
Σ	100	

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1 Intended Use

The NukEx Mag Extreme SC and NukEx Mag Extreme SL Kits are designed for rapid manual and automated 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 magnetic beads. The purified nucleic acids can be used directly as template for real time (Reverse Transcription-) PCR or any kind of enzymatic reactions.
NukEx Mag Extreme Kits allow easy automation on common liquid handling instruments or automated magnetic separators.

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 NukEx Magnetic Beads (MB).
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 NukEx Magnetic Beads with Elution Buffer (P4).

3 Components

NukEx Mag Extreme G05024-100 / G05025-100 are designed for 100 extractions.

Table 1: Components of the NukEx Mag 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 11 ml
MB	NukEx Magnetic Beads (MB)	2 x 1.0 ml
NEBC	NukEx Bead SC (incl. in G05024-100)	1x 100 vials
NEBL	NukEx Bead SL (incl. in G05025-100)	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

Note: consumables not included in the kit are dependent of the mode of sample preparation, e.g. manual extraction or extraction using magnetic particle processors such as KingFisher Flex.

- Homogenizer (Fast Prep, Precellys, TissueLyser or equivalent)
- Nuclease-free 1.5 or 2.0 ml microcentrifuge tube
- Separation plate for magnetic bead separation, e.g. Square-well Block (96-well block with 2.1 ml square-wells)
- Elution plate for collecting purified nucleic acids, e.g. Elution Plate V-bottom (96-well microtiterplate with 0.3 ml u-bottom wells)
- Pipets with sterile pipet filter tips or Tip Comps (e.g. KingFisher 96tip comb for DW magnets)
- 250 ml screw cap vial (e.g. Sarstedt Art. No. 75.9922.534)
- absolute ethanol
- distilled water (sterile Aqua dest.)
- Tabletop microcentrifuge capable of 13,000 x g centrifugal force
- Thermoblock or laboratory furnace
- Magnetic Particle Processor or magnetic separator
- 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.
- Samples must always be regarded as potentially infectious material and all equipment used has to be treated as potentially contaminated.
- Buffers contain 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 Mag Extreme Solutions.

Label	Preparation G05024-100/ G05025-100	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 accordinglyl.	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 11.1
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 Handling of Beads

A homogeneous distribution of the magnetic beads to the individual wells of the separation plate is essential for a high well-to-well consistency. Therefore, before distributing the beads, make sure that the beads are completely resuspended. Shake the storage vial briefly or place it on a vortexer.

11 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). Alternatively, the Control-DNA/-RNA or IPC can be added to the reaction vial together with the magnetic beads.

11.1 Extraction 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., 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 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 magnetic beads (shown in 11.3 or 11.4).

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 magnetic beads (shown in 11.3 or 11.4).

11.2 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 with magnetic beads (shown in 11.3 or 11.4).

11.3 Protocol for Manual Use

Step 1

- Add **20 µl NukEx Magnetic Beads (MB)** to the reaction tube.

Step 2

- Transfer **800 µl of the lysate** (supernatant) to the reaction tubes.
- Incubate for 10 min at room temperature with shaking (optional mix by pipetting up and down).
- Separate the magnetic beads against the side of the tubes by placing the tubes on a magnetic separator. Wait at least 30 seconds until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting. Do not disturb the attracted beads while aspirating the supernatant.

Step 3

- Remove the tubes from the magnetic separator.
- Add **500 µl Inhibitor Removal Buffer (P2)** and resuspend the beads by shaking (optional mix by pipetting up and down) until the beads are resuspended completely.
- Separate the magnetic beads against the side of the tubes by placing the tubes on a magnetic separator. Wait at least 30 sec until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting. Do not disturb the attracted beads while aspirating the supernatant.

Step 4

- Remove the tubes from the magnetic separator.
- Add **450 µl Wash Buffer (P3)** and resuspend the beads by shaking (optional mix by pipetting up and town) until the beads are resuspended completely.
- Separate the magnetic beads against the side of the tubes by placing the tubes on a magnetic separator. Wait at least 30 sec until all the beads have been attracted to the magnets.
- Remove and discard supernatant by pipetting. Do not disturb the attracted beads while aspirating the supernatant.

Step 5

- Remove the tubes from the magnetic separator.
- Add **450 µl Wash Buffer (P3)** and resuspend the beads by shaking (optional mix by pipetting up and town) until the beads are resuspended completely.
- Separate the magnetic beads against the side of the tubes by placing the tubes on a magnetic separator. Wait at least 30 sec until all the beads have been attracted to the magnets.
- Remove and discard supernatant by pipetting. Do not disturb the attracted beads while aspirating the supernatant.

Step 6

- Air-dry the magnetic bead pellet for 5-10 min at room temperature.

Step 7

- Remove the tubes from the magnetic separator.
- Add **100 µl Elution Buffer (P4)** and resuspend the beads by shaking (optional mix by pipetting up and town) until the beads are resuspended completely.
- Incubate for 10 min at room temperature with shaking.
- Separate the magnetic beads against the side of the tubes by placing the tubes on the magnetic separator. Wait at least 30 sec until all the beads have been attracted to the magnets.
- The supernatant contains purified nucleic acid.
- Transfer the supernatant to fresh nuclease-free tubes.

11.4 Protocol for KingFisher Flex Magnetic Particle Processor

Step 1 - Prepare wash plates

- Add **500 µl Inhibitor Removal Buffer (P2)** to each well of a Square-well Block.
- Add **450 µl Wash Buffer (P3)** to each well of a second Square-well Block.
- Add **450 µl Wash Buffer (P3)** to each well of a third Square-well Block.

Step 2 - Prepare elution plate

- Add **100 µl Elution Buffer (P4)** to each well of a Square-well Block.

Step 3 – Sample Preparation

- Add **20 µl NukEx Magnetic Beads (MB)** to the wells of a Square-well Block.
- Add 800 µl of the lysate (supernatant).

Step 4 – Run purification protocol on instrument

- Insert plates as indicated on the KingFisher™ Flex Magnetic Particle Processor.
- Method starts with a mixing step (combined lysis and binding step) after setting up the last plate to the instrument.

Step 5 – Remove eluted nucleic acid

- The instrument stops after the final elution step. Follow the instructions on the instruments display and unload the plates from the instrument.
- Purified nucleic acid can be used for further PCR based analysis.

For the KingFisher™ Flex Magnetic Particle Processors use the settings profile shown in Table 4 and Table 6.

Table 4: Reagent Information

Tip plate	Micotiter DW 96 plate	
Sample		
Name	*Well volume [µl]*	*Type*
Extraction Control	See Instruction Manual of the respective PCR Kit.	Reagent
Sample	800	Sample
Binding		
Name	*Well volume [µl]*	*Type*
Magnet Beads	20	Reagent
Inhibitor Removal Buffer	Inhibitor Removal	
Name	*Well volume [µl]*	*Type*
Inhibitor Removal	500	Reagent
1st Wash Buffer	Inhibitor Removal	
Name	*Well volume [µl]*	*Type*
Wash Buffer	450	Reagent
2nd Wash Buffer	2nd Wash Buffer	
Name	*Well volume [µl]*	*Type*
Wash Buffer	450	Reagent
Elution	Elution	
Name	*Well volume [µl]*	*Type*
Elution Buffer	100	Reagent

Table 5: Instrument Settings

	Tip 1		96 DW tip comb	
		Pick-Up	Tip plate	
		Magnetic Beads	Binding	
		Beginning of step	Precollect	No
			Release beads	Yes
		Mixing / heating	Mixing time, speed	00:10:00, Bottom mix
			Heating during mixing	No
			Heating temperature [°C]	No
		End of step	Postmix	No
			Collect count	4
			Collect time [s]	3
		Inhibitor Removal Buffer	Inhibitor Removal	
		Beginning of step	Precollect	No
			Release time, speed	00:00:30, Medium
		Mixing / heating	Shake 1 time, speed	00:00:30, Bottom mix
			Shake 2 time, speed	00:00:30, Half mix
			Heating during mixing	No
		End of step	Postmix	No
			Collect count	4
			Collect time [s]	3
		1st Wash Buffer	1st Wash Buffer	
		Beginning of step	Precollect	No
			Release time, speed	00:00:30, Medium
		Mixing / heating	Shake 1 time, speed	00:00:30, Bottom mix
			Shake 2 time, speed	00:00:30, Half mix
			Heating during mixing	No
		End of step	Postmix	No
			Collect count	3
			Collect time [s]	2
		2nd Wash Buffer	2nd Wash Buffer	
		Beginning of step	Precollect	No
			Release time, speed	00:00:30, Medium
		Mixing / heating	Mixing time, speed	00:01:00, Bottom mix
			Heating during mixing	No
		End of step	Postmix	No
			Collect count	3
			Collect time [s]	2
		Bead Drying		
			Dry time	00:05:00
			Tip position	Outside well / tube
		Elution	Elution	
		Beginning of step	Precollect	No
			Release time, speed	00:00:30, Fast
		Mixing / heating	Mixing time, speed	00:10:00, Slow
			Heating temperature [°C]	56
			Preheat	Yes
		End of step	Postmix	No
			Collect count	5
			Collect time [s]	4
		Leave	Tip plate	

12 Troubleshooting

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

Low nucleic acid yield	
Insufficient elution buffer volume	Beads pellet must be covered completely with elution buffer.
Aspiration of attracted bead pellet	Do not disturb the attracted beads while aspirating the supernatant, especially when the magnetic bead pellet is not visible in the lysate.
Aspiration and loss of beads	Time for magnetic separation too short or aspiration speed too high.
Insufficient washing procedure	Use only the appropriate combinations of separator and plates. Make sure that beads are re-suspended completely during the washing procedure. If shaking is not sufficient to resuspend the beads completely mix by repeated pipetting up and down.
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.
Time for magnetic separation too short	Increase separation time to allow the beads to be completely attracted to the magnetic pins before aspirating any liquid from the well.
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.
Reagents and samples not completely mixed	Always mix the sample tube well after addition of each reagent.

13 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)
MAGNETIC BEADS MB	NukEx Magnetic Beads (MB)
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

14 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.