Instruction for Use

NukEx Mag Extreme SC
NukEx Mag Extreme SL

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

For isolation of nucleic acids using bead beating.

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 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 Mag Extreme SC or TissueLyser - NukEx Mag Extreme SL) for mechanical disruption of samples and subsequent extraction of nucleic acids with KingFisher™ Flex Magnetic Particle Processor or other magnetic separation systems. 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.

NukEx Mag Extreme Kits allow easy automation on common liquid handling instruments or automated magnetic separators. The actual processing time depends on the configuration of the instrument and the magnetic separation system used.

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. For binding of nucleic acids to the paramagnetic beads, NukEx Magnetic Beads are added to the lysate.

After magnetic separation, the paramagnetic beads are washed to remove contaminants and salts using Inhibitor Removal Buffer and Wash Buffer. Residual ethanol from previous wash steps is removed by airdrying. 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 NukEx Magnetic Beads (MB).
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 Magnetic Beads with the Elution Buffer (P4).

3 Components

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

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

For use of NukEx Mag Extreme SC the use of FastPrep- or Precellys homogenizers and the KingFisher™ Flex Magnetic Particle Processor is recommended. For use of NukEx Mag Extreme SL the use of TissueLyser homogenizer and the KingFisher™ Flex Magnetic Particle Processor is recommended. The kits can also be used with other common homogenizers and magnetic bead separators.
Note: consumables not included in the kit are dependent of the mode of sample preparation, e.g. manual extraction or extraction using extraction robots such as KingFisher™ Flex Magnetic Particle Processors. Therefore the customer needs to decide, which consumables are necessary for his extraction process.

- Homogenizer (e.g. FastPrep® Cell Disruptor, Precellys, TissueLyser, or equivalent)
- Nuclease-free 1.5 or 2.0 ml microcentrifuge tube
- Separation plate for magnetic beads 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)
- Tabletop microcentrifuge capable of 13,000 x g centrifugal force
- absolute ethanol
- distilled water (sterile A. d.)
- Thermoblock or laboratory furnace (for isolation of DNA only)
- KingFisher™ Flex Magnetic Particle Processor or magnetic separator
- Laboratory equipment according to national safety instructions.

5 Transport, Storage and Stability

The NukEx Mag 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 Mag Extreme Kits are always shipped at +18 to +25°C.

6 General Information

6.1 Important Notes

- The NukEx Mag 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 Mag 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 Mag Extreme Solutions.

Label	Reconstitution/ Preparation G05024-100/ G05025-100	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 10.2.1.	Prepare a suspension in 2.5 ml sterile A.d., 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 Mag Extreme Kit with the KingFisher™ Flex Magnetic Particle Processor.

	DNA/ RNA
Total time	approx. 40 minutes
Hands-on time	less than 10 minutes

10 Procedure

Using NukEx Mag Extreme, RNA and DNA are extracted from sample material. Long term storage of extracted nucleic acids is recommended at ≤-65 °C.

10.1 Handling of Beads

Distribution 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 re-suspended. Shake the storage bottle well or place it on a vortexer shortly. During automation, a premix step before aspirating the beads / extraction buffer mixture from the reservoir is recommended to keep the beads re-suspended.

Magnetic Separation Time

Attraction of the magnetic beads to the magnetic pins depends on the magnetic strength of the magnetic pins, the selected separation plate, distance of the separation plate from the magnetic pins, and the volume to be processed. The individual times for complete attraction of the beads to the magnetic pins should be checked and adjusted on each system. It is recommended using the separation plates or tubes specified by the supplier of the magnetic separator.

Washing the Beads

Washing the beads can be achieved by shaking or mixing. In contrast to mixing by pipetting up and down, mixing by shaker or magnetic mixing allows simultaneous mixing of all samples. This reduces the time and number of tips needed for the preparation. Re-suspension by pipetting up and down, however, is more efficient than mixing by a shaker or magnetic mix.

10.2 Isolation of DNA and RNA

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 along with the magnetic beads.

10.2.1 Isolation of RNA and DNA 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 A.d. (dilution (w/v) / 2.5); e.g. 3 g with 7.5 ml sterile A.d. or 5 g with 12.5 ml sterile A.d. or 10 g with 25 ml sterile A.d. 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 A.d. (see page 10).

Procedure for feces

- Add 2.5 ml (or x-fold) sterile A.d. 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 10.3 or 10.4.).

Procedure for sock samples (according to FLI procedure*)

- *Cut socks into small pieces using a sterile scissor, scalpel or punch.
- *Transfer 3 g – 5 g (+/- 0.2 g) into a 50 ml reaction tube.
- *Add 7-fold volume sterile A.d., e.g. 3 g and 20 ml sterile A.d.
- *Close vial and vortex thoroughly.
- *Let the sample settle for 10 min – 20 min at RT, (alternatively: centrifugation for 5 min at 1,500 x g, RT)
- *Transfer 10 ml of the supernatant into a 15 ml or 50 ml reaction tube.
- *Centrifuge for 5 min at 4,500 x g, RT
- *Discard supernatant.
- *Resuspend the pellet in 2.0 ml sterile A.d.
- Transfer the solution 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 magnetic beads (shown in 10.3 or 10.4.).

10.2.2 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 with magnetic beads (shown in 10.3 or 10.4.).

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

For cuts: purge colonies from the agar surface using 1 ml sterile A.d.
For colony-suspensions: pick colonies from the agar surface and re-suspend in 1 ml sterile A.d.
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 magnetic beads (shown in 10.3 or 10.4.).

10.3 Detailed Protocol for Manual Use

This protocol is for manual use and serves as a guideline for adapting the kit to robotic instruments.

Step 1

- Transfer 800 µl of the lysate (supernatant) to the wells of a Square-well Block.

Step 2

- Add **20 µl NukEx Magnetic Beads (MB)** to the lysate.
- Mix immediately.
- 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 wells by placing the Square-well Block 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 Square-well Block from the magnetic separator.
- Add **500 µl Inhibitor Removal Buffer (P2)** and re-suspend the beads by shaking (optional mix by pipetting up and down) until the beads are re-suspended completely (at least 30 seconds).
- Separate the magnetic beads against the side of the wells by placing the Square-well Block on the 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 4

- Remove the Square-well Block from the magnetic separator.
- Add **450 µl Wash Buffer (P3)** and re-suspend the beads by shaking (optional mix by pipetting up and town) until the beads are re-suspended completely (at least 30 seconds)
- Separate the magnetic beads against the side of the wells by placing the Square-well Block on the 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.

Optional: Step 4 can be repeated.*

Step 5

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

Step 6

- Remove the Square-well Block from the magnetic separator.
- Add **70-100 µl Elution Buffer (P4)**.
- Incubate for 10 min at room temperature with shaking.
- Separate the magnetic beads against the side of the wells by placing the Square-well Block on the magnetic separator. Wait at least 30 seconds until all the beads have been attracted to the magnets.
- The supernatant contains purified nucleic acid.
- Transfer the supernatant to either elution plates.

*An additional wash step with Wash Buffer (P3) can increase purity of the eluted nucleic acid.

10.4 Detailed Protocol for KingFisher™ Flex Magnetic Particle Processor

Note: Always prepare deep-well block with samples first and add reagents exactly in the order as given below.

Step 1 – Sample Preparation Part I / Lysis

- Transfer 800 µl of the lysate (supernatant) to the wells of a Square-well Block.

Step 2 - Prepare wash plates

- Add **500 µl Inhibitor Removal Buffer (P2)** to each well of an empty 96-well deep-well block.
- Add **450 µl Wash Buffer (P3)** to each well of an empty the 96-well deep-well block.
- Add **450 µl Wash Buffer (P3)** to each well of a second empty the 96-well deep-well block.

Step 3 - Prepare elution plate

- Add **70-100 µl Elution Buffer (P4)** to each well of an empty 96-well deep-well block.

Step 4 – Sample Preparation Part II / Binding

- Add 20 µl NukEx Magnetic Beads to the lysate.

Step 5 – 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 6 – Remove eluted RNA/DNA

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

For the KingFisher™ Flex Magnetic Particle Processors use the settings profile shown in Table 5 and Table 6.

Table 5: 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	70-100	Reagent

Table 6: 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	2nd Wash Buffer	
		Dry time	No 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	

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 or purity	
Insufficient elution buffer volume	Beads pellet must be covered completely with elution buffer.
Insufficient performance of elution buffer during elution step	Remove residual buffers during the separation steps completely. Remaining buffers decrease the efficiency of following wash and elution steps.
Beads dried out	Do not let the beads dry as this might result in lower elution efficiencies.
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 re-suspend 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.
Aspiration speed too high (elution step)	High aspiration speed during the elution step may cause bead carry-over. Reduce aspiration speed for elution step.

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.
Impurities not completely removed	Perform a second wash step with Wash Buffer (P3) in order to completely remove salts, proteins and other residual impurities from the bound nucleic acid.

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)
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
LOT	Batch code
CONT	Content
	Consult instructions for use