


## Instruction for Use

# NukEx Complete Mag RNA/DNA

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

For isolation of nucleic acids.

<b>REF</b>	G05021-100	G05021-200
	100	200



gerbion GmbH & Co. KG  
Remsstr. 1  
70806 Kornwestheim  
Germany  
phone: +49 7154 806 20 0  
fax: + 49 7154 806 20 29  
e-mail: info@gerbion.com  
www.gerbion.com



## Index

1	Intended Use .....	3
2	Mode of Action .....	3
3	Components .....	4
4	Equipment and Reagents to be Supplied by User .....	4
5	Transport, Storage and Stability .....	5
6	General Information .....	5
6.1	Important Notes .....	5
6.2	Precautions .....	5
6.3	Handling Requirements .....	6
6.4	Laboratory Procedures .....	6
6.5	Waste Handling .....	6
7	Preparation of Solutions .....	7
8	Sample Material .....	8
9	Preparation Time .....	9
10	Procedure .....	9
10.1	Handling of Beads .....	9
10.2	Isolation of DNA and RNA .....	10
10.3	Isolation of DNA from raw milk and mastitis milk samples .....	11
10.4	Detailed Protocol for Manual Use .....	12
10.5	Detailed Protocol for KingFisher™ Flex Magnetic Particle Processor .....	14
11	Troubleshooting .....	17
12	Quality Control .....	18
13	Abbreviations and Symbols .....	19

## 1 Intended Use

The NukEx Complete Mag RNA/DNA Kit is designed for rapid manual and automated purification of nucleic acids from a wide range of samples (e.g. human samples, veterinary samples, insects, food samples, etc.). The kit is designed for use with KingFisher™ Flex Magnetic Particle Processor or other magnetic separation systems. The purified RNA / DNA can be used directly as template for RT-PCR, PCR, or any kind of enzymatic reactions.

NukEx Complete Mag RNA/DNA Kit allows 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 polymerase chain reaction (PCR) or RT-PCR, the isolation of the analyte from various sample materials is required. To this end, the sample is lysed by incubation in a proprietary lysis buffer.

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

In the presence of a chaotropic salt, nucleic acids bind specifically to the surface of the NukEx Magnetic Beads.

After magnetic separation, the paramagnetic beads are washed to remove contaminants and salts using Inhibitor Removal Buffer (P2) and Wash Buffer (P3). Residual ethanol from previous wash steps is removed by airdrying. Finally, highly pure viral RNA / DNA is eluted with low-salt Elution Buffer. Purified viral RNA / DNA can directly be used for downstream applications.

1. Samples are lysed by incubation in NukEx Complete Mag Binding Buffer. Nucleic acids are bound to the NukEx Magnetic Beads.
2. Bound nucleic acids are washed with NukEx Complete Mag Inhibitor Removal Buffer in order to remove PCR inhibitors from the sample such as > 100 U/ml heparin.
3. Bound nucleic acids are washed with NukEx Complete Mag Wash Buffer in order to purify them from salts, proteins and other cellular impurities.
4. Purified nucleic acids are eluted from the NukEx Magnetic Beads with the NukEx Complete Mag Elution Buffer.

### 3 Components

NukEx Complete Mag RNA/DNA G05021-100 is designed for 100 isolations.

NukEx Complete Mag RNA/DNA G05021-200 is designed for 200 isolations.

Table 1: Components of the NukEx Complete Mag RNA/DNA isolation kits.

Labelling		Content	
		G05021-100	G05021-200
PV1	Binding Buffer	1 x 30 ml add 26 ml 2-propanol	2 x 30 ml add 26 ml 2-propanol
PA	Poly A	2 mg	4 mg
P2	Inhibitor Removal Buffer	1 x 33 ml add 20 ml absolute ethanol	2 x 33 ml add 20 ml absolute ethanol each
P3	Wash Buffer	2 x 10 ml add 40 ml absolute ethanol	2 x 20 ml add 80 ml absolute ethanol each
P4	Elution Buffer	1 x 10,5 ml	1 x 21 ml
MB	NukEx Magnetic Beads	2 x 1.0 ml	4 x 1.0 ml

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 Complete Mag RNA/DNA the use of the KingFisher™ Flex Magnetic Particle Processor is recommended. The kit can also be used with other common 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.

- 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 v-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
- 2-propanol
- Sample Preparation Buffer SPM for preparation of milk samples (gerbion, G07014)
- Thermoblock or laboratory furnace
- KingFisher™ Flex Magnetic Particle Processor or magnetic separator
- Laboratory equipment according to national safety instructions.

## 5 Transport, Storage and Stability

The NukEx Complete Mag RNA/DNA 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  $\leq -18^{\circ}\text{C}$  (freezer) will adversely impact nucleic acid purification when precipitates form in the solutions. Therefore, NukEx Complete Mag RNA/DNA kits are always shipped at +18 to +25°C.

Reconstituted Poly A carrier RNA solution has to be aliquoted. Aliquots stored at  $\leq -18^{\circ}\text{C}$  are stable for 12 months.

## 6 General Information

### 6.1 Important Notes

- The NukEx Complete Mag RNA/DNA Kit 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 Complete Mag Binding Buffer and Inhibitor Removal Buffer contain 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 Binding Buffer and 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 Complete Mag RNA/DNA Solutions.

Label	Reconstitution/ Preparation		Storage and Stability	Purpose
	G05021-100	G05021-200		
Poly A (PA)	Dissolve in 0.5 ml Elution Buffer and prepare 50 µl aliquots.	Dissolve in 1.0 ml Elution Buffer and prepare 50 µl aliquots.	Store aliquots at ≤18°C, stable for 12 months.	Supplement of the Binding Buffer for isolation of nucleic acids.
Binding Buffer (PV1)	Add 26 ml 2-propanol to Binding Buffer and mix well. Label and date bottle accordingly after adding 2-propanol.	Add 26 ml 2-propanol to each vial Binding Buffer and mix well. Label and date bottle accordingly after adding 2-propanol.	Store at +18 to +25°C. Stable through the date of expiry printed on the kit label.	Sample lysis
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.	Add 20 ml absolute ethanol to each vial 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 40 ml absolute ethanol to each vial Wash Buffer and mix well. Label and date bottle accordingly after adding absolute ethanol.	Add 80 ml absolute ethanol to each vial 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 sample material, such as the following:

- Human samples (tissue, stool, urine, etc.)
- Veterinary samples (tissue, raw milk, etc.)
- Insects and ticks
- Food samples (milk, drinking water)
- Environmental samples
- Plant material

Table 3: Volumes of Binding Buffer (PV1) and pre-treatment of the sample for different sample matrices.

Sample material	Volume/ Amount	Volume Binding Buffer	Pre-treatment of the sample
Stool, Feces	pea-size	500 µl	Prepare a suspension in 1.5 ml sterile Aqua dest. Vortex and briefly spin down sediments. Use 200 µl of supernatants.
Swabs		500 µl	
Liquid samples*	200 µl	500 µl	
Animal/ human tissues	≤ 30 mg	500 µl	Homogenization of tissue in Binding Buffer e.g. with NukEx TS (Cat. No. G06007)
animal / human cells	≤ 2 x 10 <sup>6</sup>	500 µl	Harvest and pellet up to 2 x 10 <sup>6</sup> cells. Resuspend pellet in Binding Buffer.
Milk	100 µl	600** µl	**Dilution of 100 µl milk sample in 600 µl working solution (500 µl PV1 supplemented with 100 µl sample preparation buffer SPM).

\*Liquid samples such as amniotic fluid, CSF, urine, water, etc.



## 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 Complete Mag RNA/DNA kit with the KingFisher™ Flex Magnetic Particle Processor.

	DNA / RNA	viral RNA
<b>Total time</b>	approx. 100 minutes	approx. 40 minutes
<b>Hands-on time</b>	less than 10 minutes	less than 10 minutes.

## 10 Procedure

Procedures below are for preparing nucleic acids from 200 µl sample volume. If larger sample volumes (up to 300 µl) or other sample matrices are to be used please refer to Table 3 for appropriate buffer volumes.

### **Samples containing precipitates must be centrifuged before purification!**

Store eluted RNA at  $\leq -65^{\circ}\text{C}$  and eluted DNA at  $\leq -18^{\circ}\text{C}$  for later analysis.

### 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 resuspended. Shake the storage bottle well or place it on a vortexer shortly. Premixing magnetic beads with the binding buffer allows easier homogenous distribution of the beads to the individual wells of the separation plate. During automation, a premix step before aspirating the beads / binding buffer mixture from the reservoir is recommended to keep the beads resuspended.

#### **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. Resuspension by pipetting up and down, however, is more efficient than mixing by a shaker or magnetic mix.

## 10.2 Isolation of DNA and RNA

Please follow this protocol if you intend to either extract genomic DNA, viral DNA and RNA, bacterial DNA or DNA and RNA simultaneously. The simultaneous isolation of DNA (bacterial or viral) and viral RNA is recommended if you intend to use eluates e.g. for multiplex real time (RT-) PCRs for the detection of both pathogens with DNA genome and RNA viruses. For the (simultaneous) isolation of DNA and/or viral RNA from liquid sample material follow the protocol below, for isolation of DNA and/or viral RNA from other types of samples or complex sample matrices please refer to the overview given in Table 3 or contact our scientists on [info@gerbion.com](mailto:info@gerbion.com).

Before starting prepare a working solution of the Binding Buffer (PV1) supplemented with reconstituted Poly A (PA) for at least one sample (N) more than required in order to compensate for frothing of the buffer.

Table 5: Preparation of the working solution for isolation of DNA and RNA.

Volume needed per sample	Mastermix working solution
500 µl Binding Buffer (PV1)	500 µl x (N+1)
4 µl Poly A (PA)	4 µl x (N+1)

*Pre-warming (56–60°C) of Elution Buffer (P4) can increase the yield of DNA.*

### 10.3 Isolation of DNA from raw milk and mastitis milk samples

#### Additional materials required:

- Sample Preparation Buffer SPM (gerbion, G07014)

Before starting, prepare a working solution of the Binding Buffer (PV1) supplemented with reconstituted Poly A (PA) and Sample Preparation Buffer SPM for at least one sample (N) more than required in order to compensate for frothing of the buffer.

Table 6: Preparation of the working solution for isolation of DNA and RNA from Milk samples.

Volume needed per sample	Mastermix working solution
500 µl Binding Buffer (PV1)	500 µl x (N+1)
4 µl Poly A (PA)	4 µl x (N+1)
100 µl Sample Preparation Buffer SPM	100 µl (N+1)

#### Protocol for Manual Use

##### Step 1

- Add 20 µl NukEx Magnetic Beads to a nuclease-free 2.0 ml microcentrifuge tube. Vortex NukEx Magnetic Beads vigorously before pipetting.
- Add 600 µl working solution, freshly prepared to each tube.
- Add 100 µl sample to each tube.
- Mix immediately.
- Perform incubation for 30 min at 60°C.
- Following the lysis incubation, centrifuge 5 sec at max. speed to collect any sample from the lysis tube lid.

**Step 2** and following see 10.4. Detailed Protocol for Manual Use. Starting with Step 2.

#### Protocol for Automated Use

##### Step 1

- Add 20 µl NukEx Magnetic Beads to each well of an empty 96 deep-well block. Vortex NukEx Magnetic Beads vigorously before pipetting.
- Add 600 µl working solution, freshly prepared, to each well.
- Add 100 µl sample to each well.

**Step 2** and following see 10.4 Detailed Protocol for Manual Use. Starting with Step 2.

#### 10.4 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

- Add 20  $\mu$ l NukEx Magnetic Beads to a nuclease-free 2.0 ml microcentrifuge tube. Vortex NukEx Magnetic Beads vigorously before pipetting.
- Add 500  $\mu$ l working solution (see table 5) freshly prepared to each tube.
- Add 200  $\mu$ l sample to each tube.
- Mix immediately.
- Perform incubation for 60 min at 60°C.
- Following the lysis incubation, centrifuge 5 sec at max. speed to collect any sample from the lysis tube lids.

##### Step 2

- 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  $\mu$ l Inhibitor Removal Buffer (P2)** and resuspend the beads by shaking (optionally mix by pipetting up and down) until the beads are resuspended completely (at least 30 seconds).
- Separate the magnetic beads against the side of the tubes by placing the tubes 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 tubes from the magnetic separator.

- Add **450 µl Wash Buffer (P3)** and resuspend the beads by shaking (optional mix by pipetting up and down) until the beads are resuspended completely (at least 30 seconds)
- Separate the magnetic beads against the side of the tubes by placing the tubes 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 tubes from the magnetic separator.
- Add 100 µl Elution Buffer (P4).
- 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 seconds until all the beads have been attracted to the magnets.
- The supernatant contains purified nucleic acid.
- Transfer the supernatant to fresh nuclease-free tubes.

\*For complex sample materials, such as raw milk or stool samples, an additional wash step with Wash Buffer (P3) can increase purity of the eluted nucleic acid.

## 10.5 Detailed Protocol for KingFisher™ Flex Magnetic Particle Processor

### Step 1

- Add 20 µl NukEx Magnetic Beads to each well of an empty 96 deep-well block. Vortex NukEx Magnetic Beads vigorously before pipetting.
- Add 500 µl working solution, freshly prepared, to each well.
- Add 200 µl sample to each well.

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

### 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 elution plate

- The instrument stops after the final elution step. Follow the instructions on the instruments display and unload the plates from the instrument.
- The eluates contain purified nucleic acids.
- For storage purposes cover the elution plate with an adhesive foil.

For the KingFisher™ Flex Magnetic Particle Processors use the settings profile shown in Table 7: Reagent Information and Table 8

Table 7: Reagent Information

Tip plate	Micotiter DW 96 plate	
Lysis/Binding		
<i>Name</i>	<i>Well volume [µl]</i>	<i>Type</i>
Magnet Beads	20	Reagent
Binding Buffer	500*	Reagent
poly A	4	Reagent
Extraction Control	See Instruction Manual of the respective PCR Kit.	Reagent
Sample	200*	Sample
Inhibitor Removal Buffer	Inhibitor Removal	
<i>Name</i>	<i>Well volume [µl]</i>	<i>Type</i>
Inhibitor Removal	500	Reagent
1st Wash Buffer	Inhibitor Removal	
<i>Name</i>	<i>Well volume [µl]</i>	<i>Type</i>
Wash Buffer	450	Reagent
2nd Wash Buffer	2nd Wash Buffer	
<i>Name</i>	<i>Well volume [µl]</i>	<i>Type</i>
Wash Buffer	450	Reagent
Elution	Elution	
<i>Name</i>	<i>Well volume [µl]</i>	<i>Type</i>
Elution Buffer	100	Reagent

**\*raw milk samples and mastitis milk samples: 600 µl working solution and 100 µl sample.**

Table 8: Instrument Settings

	Tip 1	96 DW tip comb	
	Pick-Up	Tip plate	
	Blinding	Lysis	
	Beginning of step	Pause Precollect	No
	Mixing / heating	Release beads	Yes
		Mixing time, speed	01:00:00, Bottom mix
		Heating during mixing	Yes
	End of step	Heating temperature [°C]	60
		Postmix	No
		Collect count	4
		Collect time [s]	3
	Inhibitor Removal Buffer	Inhibitor Removal	
	Beginning of step	Precollect	No
	Mixing / heating	Release time, speed	00:00:30, Medium
		Shake 1 time, speed	00:00:30, Bottom mix
		Shake 2 time, speed	00:00:30, Half mix
	End of step	Heating during mixing	No
		Postmix	No
		Collect count	4
		Collect time [s]	3
	1st Wash Buffer	1st Wash Buffer	
	Beginning of step	Precollect	No
	Mixing / heating	Release time, speed	00:00:30, Medium
		Shake 1 time, speed	00:00:30, Bottom mix
		Shake 2 time, speed	00:00:30, Half mix
	End of step	Heating during mixing	No
		Postmix	No
		Collect count	3
		Collect time [s]	2
	2nd Wash Buffer	2nd Wash Buffer	
	Beginning of step	Precollect	No
	Mixing / heating	Release time, speed	00:00:30, Medium
		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
	Mixing / heating	Release time, speed	00:00:30, Fast
		Mixing time, speed	00:10:00, Slow
		Heating temperature [°C]	56
	End of step	Preheat	Yes
		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 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](mailto: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 resuspended 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 ethanol 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 elution step may cause bead carry-over. Reduce aspiration speed for elution.

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. Aliquot Poly A (PA) after reconstitution and store aliquots at $\leq -18^{\circ}\text{C}$ .
2-propanol not added to Binding Buffer (PV1)	Add 2-propanol to the buffer before using. Mix the buffer well and store at +18 to 25°C. Always mark the buffer vial to indicate whether 2-propanol has been added or not.
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 Quality Control







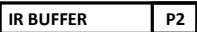









In accordance with gerbion's ISO-certified Quality Management System, each lot of the NukEx Complete Mag RNA/DNA Kit is tested against predetermined specifications to ensure consistent product quality.

Dilution series of MS2 RNA, Lambda-Phage DNA, and human genomic DNA are applied to the NukEx Magnetic Beads, washed and eluted according to the kit protocol. 4  $\mu\text{l}$  of the eluate is analyzed by real time (RT-) PCR. Recovery of at least  $2 \times 10^5$  RNA or DNA molecules per 200  $\mu\text{l}$  sample are guaranteed.

For the validation of the NukEx Complete Mag RNA/DNA Kit genomic DNA, viral RNA, viral DNA, and bacterial DNA was isolated from a wide range of sample matrices such as tissue, stool, ticks, milk, buccal swabs, plant material etc.

Eluates were used as template in real time PCR and real time RT-PCR and produced highly specific PCR products with good yields.

### 13 Abbreviations and Symbols

DNA	Deoxyribonucleic Acid		Catalog number
RNA	Ribonucleic Acid		Contains sufficient for <n> test
PCR	Polymerase Chain Reaction		Upper limit of temperature
RT	Reverse Transcriptase		Manufacturer
	Binding Buffer (PV1)		Use by YYYY-MM-DD
	Inhibitor Removal Buffer (P2)		Batch code
	Wash Buffer (P3)		Content
	Elution Buffer (P4)		Consult instructions for use
	Poly A (PA)		<i>In vitro</i> diagnostic medical device
	Nuk Ex Magnetic Beads (MB)		European Conformity