Instruction Manual

NukEx Mag RNA/DNA

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

For isolation of nucleic acids using magnetic beads.

REF	G05012-100	G05012-200
Σ	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

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1 Intended Use

The NukEx 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 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 (Binding Buffer (P1) supplemented with Proteinase K).
For binding of nucleic acids, NukEx Magnetic Beads are added to the lysate.
In the presence of a chaotropic salt, nucleic acids bind to the surface of the NukEx Magnetic Beads.
After magnetic separation, the magnetic 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 air drying. Finally, highly pure RNA / DNA is eluted with low-salt Elution Buffer (P4). Purified RNA / DNA can directly be used for downstream applications.

1. Samples are lysed by incubation in NukEx Binding Buffer (P1). Nucleic acids are bound to the NukEx Magnetic Beads.
2. Bound nucleic acids are washed with NukEx Inhibitor Removal Buffer (P2) in order to remove PCR inhibitors from the sample such as > 100 U/ml heparin.
3. Bound nucleic acids are washed with NukEx Wash Buffer (P3) 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 Elution Buffer (P4).

3 Components

NukEx Mag RNA/DNA G05012-100 is designed for 100 isolations.
NukEx Mag RNA/DNA G05012-200 is designed for 200 isolations

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

Labelling		Content	
		G05012-100	G05012-200
P1	Binding Buffer	1 x 40 ml add 17 ml 2-propanol	2 x 40 ml add 17 ml 2-propanol each
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 up 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 RNA/DNA the use of the KingFisher™ Flex Magnetic Particle Processor or equivalent instrumentation is recommended.
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.

- Proteinase K (e.g. gerbion G07001)
- 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
- 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
- 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

NukEx Mag RNA/DNA Kit components are shipped at ambient temperature. NukEx 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 ≤-18°C (freezer) will adversely impact nucleic acid purification when precipitates form in the solutions.

Reconstituted Poly A carrier RNA solution has to be aliquoted. Aliquots stored at ≤-18°C are stable for 12 months.

6 General Information

6.1 Important Notes

- The NukEx 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 and all equipment used has to be treated as potentially contaminated.

6.2 Precautions

- NukEx 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 of 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.
- 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 from gerbion upon request.

7 Preparation of Solutions

Table 2: Preparation of NukEx Mag RNA/DNA Solutions.

Label	Reconstitution/ Preparation G05012-100	G05012-200	Storage and Stability	Purpose
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 (P1)	Add 17 ml 2-propanol to Binding Buffer and mix well. Label and date bottle accordingly after adding 2-propanol.	Add 17 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 ethanol.	Add 20 ml absolute ethanol to each vial Inhibitor Removal Buffer and mix well. Label and date bottle accordingly after adding 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 ethanol.	Add 80 ml absolute ethanol to each vial Wash Buffer and mix well. Label and date bottle accordingly after adding 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 (EDTA-blood, tissue, stool, urine, etc.)
- Veterinary samples (EDTA-blood, tissue, raw milk, etc.)
- Insects and ticks
- Food samples (milk, drinking water)
- Environmental samples
- Plant material

Table 3: Volumes of Binding Buffer (P1) 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 $_dH_2O$. 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 10^6	500 µl	Harvest and pellet up to 2 x 10^6 cells. Resuspend pellet in Binding Buffer.

*Liquid samples such as EDTA-blood, serum, amniotic fluid, CSF, urine, water, milk 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 Mag RNA/DNA kit with the KingFisher™ Flex Magnetic Particle Processor.

	DNA/ RNA
Total time	approx. 50 minutes
Hands-on time	less than 10 minutes

10 Procedure

Procedures below are for preparing nucleic acids from 200 µl sample volume. If solid sample matrices are to be used please refer to Table 3 for appropriate buffer volumes. For information on the extraction from other sample matrices please contact our scientists on info@gerbion.com.

Samples containing precipitates must be centrifuged before purification!

Store eluted nucleic acid at ≤-18°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 of Beads

Washing of magnetic 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 Nucleic Acids

Before starting, prepare a working solution of the Binding Buffer (P1) supplemented with reconstituted Poly A (PA) and Proteinase K 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.

Volume needed per sample	Mastermix working solution
500 µl Binding Buffer (P1)	500 µl x (N+1)
4 µl Poly A (PA)	4 µl x (N+1)
50 µl Proteinase K [20 mg/ml]	*50 µl x (N+1)*

Pre-warming (56-60°C) of Elution Buffer (P4) can increase the yield of nucleic acids.

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

- Add **550 µl working solution**, freshly prepared to a nuclease-free 2.0 ml microcentrifuge tube.
- Add **200 µl sample** to the microcentrifuge tube.
- Mix immediately.
- Perform Proteinase K digest for 10 min at 56-60°C.
- Following the lysis incubation, centrifuge 5 sec at max. g to collect any sample from the lysis tube lids and transfer each lysate to the wells of a Square-well Block.

Step 2

- Add **20 µl Magnetic Beads** to the lysate.
- Mix immediately.
- Incubate for 10 min at room temperature with shaking (optional mix by pipetting up and town).
- 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 resuspend the beads by shaking (optional mix by pipetting up and town) until the beads are resuspended 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 resuspend the beads by shaking (optional mix by pipetting up and town) until the beads are resuspended completely (at least 30 seconds). Alternatively, resuspend beads completely by repeated pipetting up and down.
- 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 (*optional: pre-warmed [56-60°C]*) 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.

*For complex sample materials, such as whole blood or stool samples, 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

- Add 550 µl working solution, freshly prepared, to each well of an empty 96-well deep-well block.
- Add 200 µl sample to each well.
- Mix immediately.
- Perform Proteinase K digest for 10 min at 56-60°C.

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 50-100 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 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 nucleic acids

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

For the KingFisher™ Flex Magnetic Particle Processors use the settings profile shown in Table 6 and Table 7. The settings are also available as data file for direct transfer to the KingFisher™ Flex Magnetic Particle Processor.

Table 6: Reagent Information

Tip plate	Micotiter DW 96 plate	
Lysis		
Name	*Well volume [µl]*	*Type*
Binding Buffer	500	Reagent
Proteinase K	50	Reagent
poly A	4	Reagent
Extraction Control	See Instruction Manual of the respective PCR Kit.	Reagent
Sample	200	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 7: Instrument Settings

Tip 1		96 DW tip comb	
	Pick-Up	Tip plate	
	Binding Buffer	Lysis	
		Pause	
	Beginning of step	Precollect	No
		Release beads	Yes
	Mixing / heating	Mixing time, speed	00:10:00, Bottom mix
		Heating during mixing	Yes
		Heating temperature [°C]	56
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	3
	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	No00:05:00
		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	
Sample not sufficiently lysed	Supplement the working solution (=Binding Buffer + Poly A) with 50 µl Proteinase K (20 mg/ml) per sample.
Incomplete Proteinase K digestion	Be sure to dissolve the lyophilized Proteinase K completely, as follows: 1. Pipette appropriate volume of PCR grade water to lyophilised Proteinase K in order to get a concentration of 20 mg/ml (e.g. 2.5 ml PCR grade water to 50 mg Proteinase K). 2. Re-close vial and invert until all the lyophilisate (including any stuck to the lid) is completely dissolved. 3. Aliquot the reconstituted enzyme, mark each aliquot with the date of reconstitution, and store at ≤-18°C. Reconstituted Proteinase K is stable for 12 months when stored properly.
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, 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 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 Proteinase K and Poly A (PA) after reconstitution and store aliquots at ≤ -18°C.
2-propanol not added to Binding Buffer (P1)	Add 2-propanol to the buffer before using. After adding 2-propanol, mix the buffers 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.
Absorbance reading of product too high	
Poly A in the eluate	While Guanine, Cytosine, and Thymine have OD260/280 ratios of 1.1 – 1.5 when measured on their own, the ratio for Adenine is at 4.5. Poly Adenine (poly A) added to the working solution can also be found in the eluate. The OD260/280 ratios of the eluate are therefore higher than expected for pure DNA or RNA.
High levels of RNase activity	Be careful to create an RNase-free working environment.

12 Assay Validation

Extraction Control

Use the Extraction Control included in real time (RT-) PCR Kits for downstream processing. E.g. add 5 µl of the Extraction Control per reaction directly to the Binding Buffer and co-elute with the nucleic acid of the sample. The Ct value of the Extraction Control in the subsequent real time (RT-) PCR needs to meet the validation criteria of the respective real time (RT-) PCR Kit.

13 Kit Performance

The scope of the validation was to show that the performance characteristics NukEx Mag RNA/DNA and if the method meets the requirements of their intended application, that is to extract DNA and RNA from clinical and environmental samples.

During validation, NukEx Mag RNA/DNA was tested against other commercial extraction kits using standardized samples. The quality and quantity of extracted nucleic acids were determined using real time PCR and real time RT-

PCR.The extractions of nucleic acids were performed according to the manufacturers instructions.

13.1 Sample Material

Table 8: Overview of the samples tested.

Sample	Pathogens detected	Genomic DNA detected
Avian faeces	Influenzaviruses	nd
Buccal swabs	Influenzaviruses, Adenovirus, RSV	nd
Cerebrospinal fluid	Enteroviruses, TBEV	nd
Bacterial cultures	E. coli, Streptococci, Legionella, Mycobacteria incl. MTB, Salmonella, Listeria, Campylobacter, Shigella	nd
Bovine blood samples	BVDV	nd
Bovine brain samples	SBV	nd
Bovine faeces	MAP	nd
Bovine raw milk	MAP	nd
Bovine tissue samples	Coxiella burnetii (Q Fever)	yes
Drinking water	Legionella	nd
Human blood samples	CMV	yes
Human epithel	nd	yes*
Human hair with root	nd	yes*
Human muscle	nd	yes*
Human nails	nd	yes*
Human sperm	nd	yes*
Human sputum	nd	yes*
Human teeth	nd	yes*
Human urine samples	CMV	nd
Human stool samples	Norovirus, Sapovirus, Astrovirus, Rotavirus, Adenovirus, Salmonella, E. coli	nd
Ovine faeces	MAP	nd
Tissue culture samples	VZV, CMV, EBV, Enteroviruses, Polioviruses, HSV 1+2, Influenzaviruses, RSV, Rotavirus, Adenovirus, Babesia	nd
Ticks	TBEV, Borrelia, Ehrlichia, Babesia	yes

*Samples were tested in a forensic lab.

The samples were either field samples positive for pathogens (e.g. bovine faeces and milk positive for Mycobacterium avium ssp. paratuberculosis, bovine ear notch samples positive for BVD, porcine saliva positive for PRRSV, ticks positive for Borrelia and TBEV, bovine tissue samples positive for Coxiella burnetii) or sample material was artificially spiked with pathogens or, in case of forensic samples, human genomic DNA should be isolated. If spiking was done, the sample materials were spiked with the respective pathogens, natively found in this materials in infected subjects (e.g. urine spiked with Cytomegalovirus, buccal swabs spiked with Influenzaviruses, see table 5 sample matrices).

13.2 DNA Extraction

The following table shows an overview of the performance of DNA extraction (genomic, bacterial, viral) using NukEx Mag RNA/DNA (A) in comparison to competitors, indicated by characters in the first row of the table.

The +/++/+++ indicate the DNA yield and outcome of the subsequently performed real time PCR.

na = not applicable

nd = not done

Table 9: Comparison of DNA extraction efficiencies.

Sample	A	B	C	D	E	F	G	H	I	K	L	M
Avian faeces	+++	na	na	na	na	na	na	na	na	na	na	na
Buccal swabs	+++	na	+++	nd	++	na	na	na	na	++	++	na
Cerebrospinal fluid	+++	na	+++	na	+++	na	na	na	na	na	++	++
Bacterial cultures	+++	na	++	na	na	na	na	na	na	na	++	na
Bovine blood samples	+++	na	++	++	na	++	na	na	na	na	++	na
Bovine brain samples	+++	na	na	++	na	na	na	++	na	na	na	na
Bovine faeces	+++	na	++	na	na	na	++	na	na	na	na	na
Bovine raw milk	+++	na	na	na	na	na	na	na	na	na	na	na
Bovine tissue samples	+++	na	na	++	na	na	na	++	na	na	na	na
Drinking water	+++	na	+++	+++	na	+++	na	na	na	na	nd	na
Human blood samples	+++	na	+++	+++	na	+++	na	na	na	na	nd	+++
Human epithel	++	na	nd	nd	na	na	na	++	na	na	na	na
Human hair with root	++	na	nd	nd	na	na	na	+++	na	na	na	na
Human muscle	+++	na	nd	nd	na	na	na	+++	na	na	na	na
Human nails	+	na	nd	nd	na	na	na	+++	na	na	na	na
Human sperm	+++	na	nd	nd	na	na	na	++	na	na	na	na
Human sputum	+++	na	nd	nd	na	na	na	+++	na	na	na	na
Human teeth	+	na	nd	nd	na	na	na	+++	na	na	na	na
Human urine samples	+++	na	nd	na	++	na	na	na	na	na	na	na
Human stool samples	+++	+++	nd	na	na	na	na	na	na	na	na	na
Ovine faeces	++	na	na	na	na	na	na	na	na	na	na	na
Tissue culture samples	+++	na	na	nd	na	na	na	++	na	na	na	na
Ticks	+++	na	na	nd	na	na	na	++	na	na	na	na

The results shown in Table 9 indicate, that NukEx Mag RNA/DNA can be used for the extraction of DNA from a variety of different sample materials. For the extraction of genomic DNA from human nails and teeth, a bead-beating step before using NukEx Mag RNA/DNA is recommended. The results shown for

these materials are without bead-beating prior to extraction. Furthermore, for the extraction of Mycobacteria DNA from faeces and sputum, and RNA and DNA from ticks, a bead-beating or other mechanical disruption pre-extraction treatment is also highly recommended.

13.3 RNA Extraction

The following table shows an overview of the performance of viral RNA extraction using NukEx Mag RNA/DNA (A) in comparison to competitors, indicated by characters in the first row of the table.

The +/++/+++ indicate the RNA yield and outcome of the subsequently performed real time RT-PCR.

na = not applicable

nd = not done

Table 10: Comparison of RNA extraction efficiencies.

Sample	A	B	C	D	E	F	G	H	I	K	L	M
Avian faeces	+++	+++	na	na	na	na	na	na	na	na	na	na
Buccal swabs	+++	+++	na	na	na	na	na	na	na	na	na	++
Cerebrospinal fluid	+++	+++	nd	na	na	na	na	na	na	++	na	++
Bovine blood	+++	++	++	na	na	na	na	na	na	++	na	++
Bovine brain samples	+++	++	na	na	na	na	na	na	++	na	na	na
Bovine faeces	+++	na	++	na	na	na	na	na	na	na	na	na
Bovine raw milk	+++	na	na	na	na	na	na	na	na	na	na	na
Bovine tissue	+++	++	na	na	na	na	na	na	na	na	na	na
Human urine	+++	na	nd	na	na	na	na	na	na	na	na	na
Human stool	+++	+++	nd	na	na	na	++	na	na	na	na	na
Ovine faeces	++	na	na	na	na	na	na	na	na	na	na	na
Tissue culture	+++	+++	na	nd	na	na	na	na	++	na	na	na
Ticks	+++	na	na	nd	na	na	na	na	++	na	na	na

The results shown in Table 10 indicates, that NukEx Mag RNA/DNA can be used for the extraction of RNA from a variety of different sample materials. For the extraction of RNA from ticks, a bead-beating or other mechanical disruption pre-extraction treatment is also highly recommended.

14 Abbreviations and Symbols

DNA	Deoxyribonucleic Acid	REF	Catalog number
RNA	Ribonucleid Acid	Σ	Contains sufficient for <n> test
PCR	Polymerase Chain Reaction	+25°C +18°C	Limit of temperature
RT	Reverse Transcription		Manufacturer
CMV	Cytomegalovirus		Use by YYYY-MM
MAP	Mycobacterium avium ssp paratubeculosis	LOT	Batch code
VZV	Varicella Zoster Virus	CONT	Content
EBV	Epstein Barr Virus		Consult instructions for use
HSV	Herpes Simplex Virus	SBV	Schmallenberg Virus
RSV	Respiratory Syncytial Virus	IVD	*In vitro* diagnostic medical device
BINDING BUFFER P1	Binding Buffer (P1)	ELUTION BUFFER P4	Elution Buffer (P4)
IR BUFFER P2	Inhibitor Removal Buffer (P2)	POLY A PA	Poly A (PA)
WASH BUFFER P3	Wash Buffer (P3)	MAGNETIC BEADS MB	Magnetic Beads

The product has been classified and marked in accordance with EU Directives / Ordinance on Hazardous Materials.

Acute toxicity, Category 4, H302
Acute toxicity, Category 4; H332
Skin irritation, Category 2; H315
Eye irritation, Category. 2; H319

15 Literature

[1] Sambrook, J. and Russell, D.W.: Molecular Cloning, 2001.