Instruction for Use

NukEx Complete RNA/DNA

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

For isolation of nucleic acids.

REF	G05020-50	G05020-200
Σ	50	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 Complete RNA/DNA Kit is designed for the purification of nucleic acids from a wide range of difficult samples (e.g. human and veterinary samples, insects, etc.). The kit can be used especially for the extraction of nucleic acids from difficult matrices (e.g. bovine feces or feces from small ruminants). The purified nucleic acids are applied in PCR or RT-PCR directly after elution in PCR grade water.

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. In the presence of a chaotropic salt, nucleic acids bind specifically to the surface of the glass fibres within the NukEx Spin Columns. Specifically bound to the glass fibres, the nucleic acids can be purified from salts, proteins and other components found in the sample by washing with the NukEx Complete Inhibitor Removal and Wash Buffers.

1. Samples are lysed by incubation in NukEx Complete Binding Buffer. Nucleic acids are bound to the glass fibres within the NukEx Spin Columns.
2. Bound nucleic acids are washed with NukEx Complete 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 Wash Buffer in order to purify them from salts, proteins and other cellular impurities.
4. Purified nucleic acids are eluted from the NukEx Spin Columns with the NukEx Complete Elution Buffer.

3 Components

NukEx Complete RNA/DNA G05020-50 is designed for 50 isolations.
NukEx Complete RNA/DNA G05020-200 is designed for 200 isolations

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

Labelling		Content	
		G05020-50	G05020-200
PV1	Binding Buffer	1 x 21 ml add 9 ml 2-propanol	2 x 40 ml add 17 ml 2-propanol
PA	Poly A	1 mg	4 mg
P2	Inhibitor Removal Buffer	1 x 16.5 ml add 10 ml absolute ethanol	2 x 33 ml add 20 ml absolute ethanol each
P3	Wash Buffer	1 x 10 ml add 40 ml absolute ethanol	2 x 20 ml add 80 ml absolute ethanol each
P4	Elution Buffer	1 x 4.5 ml	1 x 12 ml
	NukEx Spin Columns	50 pieces	200 pieces

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

- Laboratory equipment according to national safety instructions.
- PCR grade water
- Sterile pipet tips with filter
- Nuclease-free Collection Tubes (gerbion G06008)
- Nuclease-free 1.5 ml or 2.0 ml microcentrifuge tube
- Tabletop microcentrifuge capable of 13,000 x g centrifugal force
- Absolute ethanol
- 2-propanol
- Thermoblock or laboratory furnace (for isolation of DNA only)

5 Transport, Storage and Stability

The NukEx Complete 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 -15 to -25°C (freezer) will adversely impact nucleic acid purification when precipitates form in the solutions. Therefore, NukEx Complete RNA/DNA kits are always shipped at +18 to +25°C.
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 Complete 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 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 RNA/DNA Solutions.

Label	Reconstitution/ Preparation G05020-50	G05020-200	Storage and Stability	Purpose
Poly A (PA)	Dissolve in 0.25 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 9 ml 2-propanol 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 10 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 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 (PV1) and pre-treatment of the sample for different sample matrices.

Sample Material	Volume/ Amount	Volume Binding Buffer	Pre-treatment of the Sample
Faeces	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	
Tissue	≤ 30 mg	500 µl	Homogenization of tissue in Binding Buffer e.g. with NukEx TS (Cat. No. G06007)
Tissue	≤2 x 10^6 cells	500 µl	Harvest and pellet up to 2 x 10^6 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 EDTA-blood, serum, 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 RNA/DNA kit.

	DNA/ RNA	viral RNA
Total time	approx. 70 minutes	approx. 10 minutes
Hands-on time	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 ≤-65 °C and eluted DNA at ≤-18 °C for later analysis.

11 Before You Begin

11.1 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.

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 (60°C) of Elution Buffer (P4) can increase the yield of DNA.

Step 1

- Add **200 µl sample** to a nuclease-free 2.0 ml microcentrifuge tube.
- Add **500 µl working solution**, freshly prepared.
- Mix immediately.
- For DNA/RNA isolation incubate for 60 min at 60°C. If only viral RNA should be extracted, the step can be omitted.

Step 2

- Pipet entire mixture into the reservoir of the NukEx Spin Column.
- Centrifuge 1 min at 8,000 × g.
- Remove the NukEx Spin Column from the Collection Tube, discard the flowthrough liquid and optionally the Collection Tube.
- Optionally, replace the Collection Tube.

Step 3

- Add **500 µl Inhibitor Removal Buffer (P2)** into the reservoir of the NukEx Spin Column.
- Centrifuge 30 s at 8,000 × g.
- Remove the NukEx Spin Column from the Collection Tube, discard the flowthrough liquid and optionally the Collection Tube.
- Optionally, replace the Collection Tube.

Step 4

- Add **450 µl Wash Buffer (P3)** into the reservoir of the NukEx Spin Column.
- Centrifuge 30 s at 8,000 × g.
- Remove the NukEx Spin Column from the Collection Tube, discard the flowthrough liquid and optionally the Collection Tube.
- Optionally, replace the Collection Tube.
- *Optional*: Add **450 µl Wash Buffer (P3)** into the reservoir of the NukEx Spin Column.*
- *Centrifuge 30 s at 8,000 × g.*
- Centrifuge 10 s at maximum speed (13,000 × g) in order to completely remove the ethanol from the column.

Step 5

- Transfer the NukEx Spin Column into a nuclease-free 1.5 ml microcentrifuge tube.
- Add **20-50 µl (*optional: pre-warmed [60°C]*) Elution Buffer (P4)** into the reservoir of the NukEx Spin Column.
- *Optional: Incubate for 1 min at room temperature.*
- Centrifuge 1 min at 8,000 × g.
- The eluate contains purified nucleic acid.

* For complex sample materials, such as whole blood or faeces, an additional wash step with Wash Buffer (P3) can increase purity of the eluated nucleic acid.

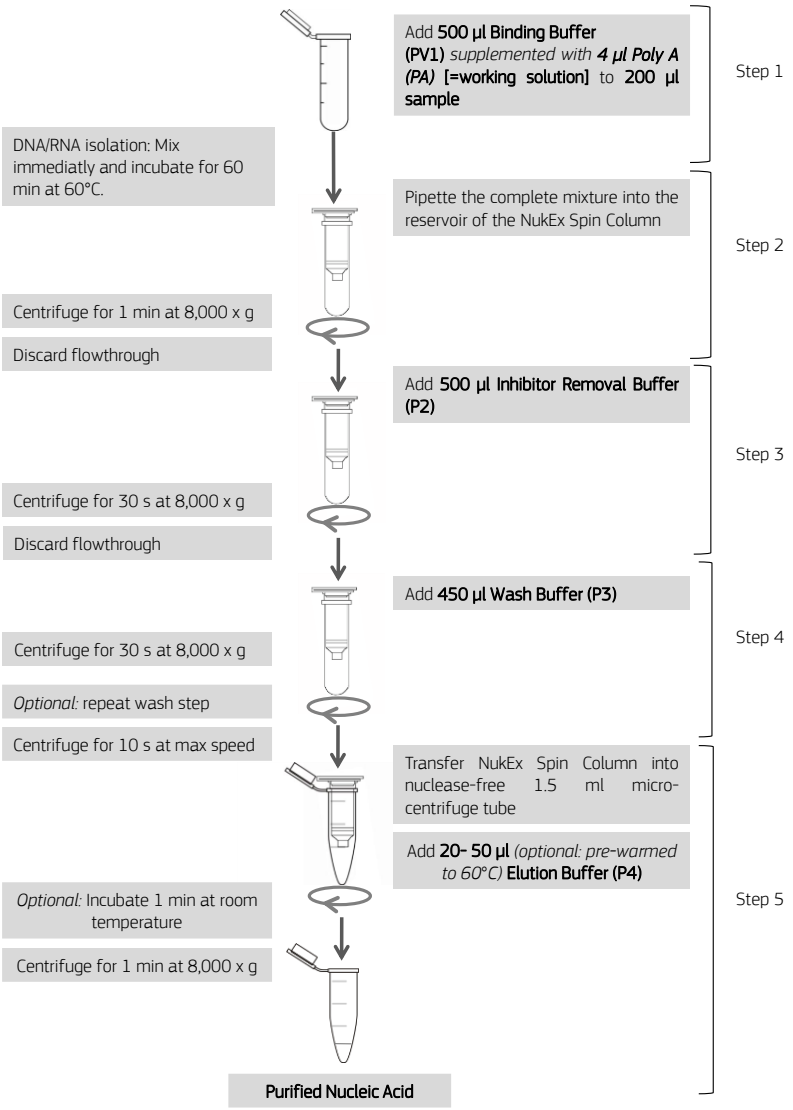

Figure 1: Protocol for the (simultaneous) Isolation of DNA and/or viral RNA.

11.2 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)

Step 1

- Add 600 µl working solution, freshly prepared to a nuclease-free 2.0 ml microcentrifuge tube.
- Add 100 µl sample to each tube.
- Mix immediately.
- Perform digest for 30 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 and following see 11.1 Isolation of DNA and RNA.

12 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 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	
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 ≤-18°C.
2-propanol not added to Binding Buffer (PV1)	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.
Poor elution of nucleic acids with water	
Water has wrong pH	If you use your own water or buffer to elute nucleic acids from the NukEx Spin Column, be sure it has the same pH as the Elution Buffer supplied in the kit.

13 Quality Control

In accordance with gerbion's ISO-certified Quality Management System, each lot of the NukEx Complete 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 Spin Columns, washed and eluted according to the kit protocol. 4 µl of the eluate is analyzed by real time (RT-) PCR. Recovery of at least 2 x 10^5 RNA or DNA molecules per 200 µl sample are guaranteed.
For the validation of the NukEx Complete RNA/DNA Kit viral RNA, genomic DNA, viral DNA, and bacterial DNA was isolated from a wide range of sample matrices such as blood, tissue, feces, ticks, milk, buccal swabs etc.

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

14 Abbreviations and Symbols

DNA	Deoxyribonucleic Acid	REF	Catalog number
RNA	Ribonucleic Acid	Σ	Contains sufficient for <n> test
PCR	Polymerase Chain Reaction	+25°C +18°C	Upper limit of temperature
RT	Reverse Transcriptase		Manufacturer
BINDING BUFFER PV1	Binding Buffer (PV1)		Use by YYYY-MM
IR BUFFER P2	Inhibitor Removal Buffer (P2)	LOT	Batch code
WASH BUFFER P3	Wash Buffer (P3)	CONT	Content
ELUTION BUFFER P4	Elution Buffer (P4)		Consult instructions for use
POLY A PA	Poly A (PA)	IVD	*In vitro* diagnostic medical device
SPIN COLUMNS	Spin Columns	CE	European Conformity