

Instruction for Use

NukEx Pure RNA/DNA

For extraction of nucleic acids using columns.

REF	G05004-100	G05004-200
	100	200



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Carrier RNA solution has been replaced by PolyA/Carrier RNA lyophilized powder.



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1 Intended Use

NukEx Pure RNA/DNA Kit is designed for rapid manual purification of nucleic acids from a wide range of samples (e.g. human samples, veterinary samples, insects, food samples, etc.). Each IVD application of this sample preparation procedure in combination with an in vitro diagnostic downstream nucleic acid test must be evaluated for the respective IVD parameter.

2 Mode of Action

- a) Samples are lysed by incubation in Working Solution (Binding Buffer (P1) substituted with 2-propanol). Nucleic acids are bound to the glass fibres within the Spin Columns.
- b) Bound nucleic acids are washed with Inhibitor Removal Buffer (P2) in order to remove PCR inhibitors from the sample.
- c) Bound nucleic acids are washed with Wash Buffer (P3) in order to purify them from salts, proteins and other cellular impurities.
- d) Purified nucleic acids are eluted from the Spin Columns with the Elution Buffer (P4).

Purified nucleic acids can be used directly for downstream applications.

3 Components

NukEx Pure RNA/DNA G05004-100 is designed for 100 isolations.

NukEx Pure RNA/DNA G05004-200 is designed for 200 isolations.

Table 1: Components of the NukEx Pure RNA/DNA extraction kits.

Labelling		Content	
		G05004-100	G05004-200
P1	Binding Buffer	1 x 30 ml, add 26 ml 2-propanol	2 x 30 ml, add 26 ml 2- propanol, each
PA	PolyA/Carrier RNA	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	1 x 20 ml, add 80 ml absolute ethanol	2 x 20 ml, add 80 ml absolute ethanol, each
P4	Elution Buffer	1 x 6 ml	1 x 12 ml
	Spin Columns	100 pieces	200 pieces
	Collection Tubes	100 pieces	200 pieces

All solutions are clear and should not be used when precipitates have formed. Warm up 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
- Proteinase K (e.g. gerbion G07001 or G07019)
- Sterile pipet tips with filter
- Additional Nuclease-free Collection Tubes (gerbion G06008)
- Nuclease-free 1.5 ml or 2.0 ml microcentrifuge tubes
- Tabletop microcentrifuge capable of 13,000 x g centrifugal force
- Absolute ethanol
- 2-propanol
- Thermoblock or laboratory furnace

5 Transport, Storage and Stability

NukEx Pure RNA/DNA Kit components are shipped at ambient temperature. Kits must be stored at +18 to +25°C. If properly stored, all kit components are stable until the date of expiry printed on the label.

Please note that improper storage at +2 to +8°C (refrigerator) or ≤-18°C (freezer) will adversely impact nucleic acid purification when precipitates form in the solutions. Reconstituted PolyA/Carrier RNA solution has to be aliquoted. Aliquots stored at ≤-18°C are stable through date of expiry printed on kit label.

6 General Information

- The NukEx Pure 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.
- Binding Buffer (P1) and Inhibitor Removal Buffer (P2) contain guanidine hydrochloride which is an irritant. Always wear gloves and follow standard safety precautions.
- Do not let these buffers touch your skin, eyes, or mucous membranes. If contact does occur, wash the affected area immediately with large amounts of water; otherwise, the reagent may cause burns. If you spill the reagent, dilute the spill with water before wiping it up.
- Always wear gloves and follow standard safety precautions when handling these buffers.

- 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.

6.1 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 Pure RNA/DNA Solutions.

Label	Preparation		Storage and Stability
	G05004-100	G05004-200	
PolyA/ Carrier RNA (PA)	Dissolve in 0.5 ml Elution Buffer and prepare 50 µl aliquots.	Dissolve in 1 ml Elution Buffer and prepare 50 µl aliquots.	Store at ≤-18°C. Stable through date of expiry printed on kit label.
Binding Buffer (P1)	Add 26 ml 2-propanol, mix well. Label and date bottle accordingly.	Add 26 ml 2-propanol to each vial, mix well. Label and date bottle accordingly.	Store at +18 to +25°C. Stable through date of expiry printed on kit label.
Inhibitor Removal Buffer (P2)	Add 20 ml absolute ethanol, mix well. Label and date bottle accordingly.	Add 20 ml absolute ethanol to each vial, mix well. Label and date bottle accordingly.	
Wash Buffer (P3)	Add 80 ml absolute ethanol, mix well. Label and date bottle accordingly.	Add 80 ml absolute ethanol to each vial, mix well. Label and date bottle accordingly.	

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), pre-treatment for various 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 PCR-grade water Vortex and briefly spin down sediments. Use 200 µl of the supernatant.
Swabs		500 µl	Add 500 µl PCR-grade water to a dry swab, suspend vigorously and use 200µl of the suspension.
Liquid samples*	200 µl	500 µl	
Tissues	≤ 30 mg	500 µl	Homogenization of tissue in 500 µl PCR-grade water e.g. with NukEx TS (gerbion, Cat. No. G06007) using NukEx Pestle (gerbion, Cat. No. G06006) or tissue homogenizer (e.g. Precellys, Bertin Instruments). Spin down for 1 min. at 8,000 x g. Use 200 µl of the supernatant.
Cells	≤ 2 x 10 ⁶	500 µl	Harvest and pellet up to 2 x 10 ⁶ cells. Resuspend pellet in 200 µl PCR-grade water. Homogenization e.g. with NukEx TS (gerbion, Cat. No. G06007) using NukEx Pestle (gerbion, Cat. No. G06006) or tissue homogenizer (e.g. Precellys, Bertin Instruments). Spin down for 1 min. at 8,000 x g. Use 200 µl of the supernatant.

*Liquid samples such as EDTA-blood, serum, amniotic fluid, CSF, urine, water, milk etc.

Samples containing precipitates must be centrifuged before purification!

Store eluted nucleic acid at $\leq -18^{\circ}\text{C}$ for later analysis.

9 Extraction of Nucleic Acids

Before starting, prepare a working solution of the Binding Buffer (P1) supplemented with PolyA/Carrier RNA (PA) and Proteinase K for at least one sample (N) more than required in order to compensate pipetting inaccuracies.

Table 4: Preparation of the working solution.

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

Step 1

- Add **550 μl working solution**, freshly prepared, to a nuclease-free 2.0 ml microcentrifuge tube.
- Add **200 μl sample**.
- Mix immediately.
- Incubate for 10 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

- Pipet entire mixture into the reservoir of the Spin Column.
- Centrifuge 1 min at $8,000 \times g$.
- Remove the Spin Column from the Collection Tube, discard the flowthrough liquid and the Collection Tube.
- Replace the Collection Tube.

Step 3

- Add **500 μl Inhibitor Removal Buffer (P2)** into the reservoir of the Spin Column.
- Centrifuge 30 sec at $8,000 \times g$.
- Remove the Spin Column from the Collection Tube, discard the flowthrough liquid and the Collection Tube.
- Replace the Collection Tube.

Step 4

- Add **450 µl Wash Buffer (P3)** into the reservoir of the Spin Column.
- Centrifuge 30 sec at 8,000 × g.
- Remove the Column from the Collection Tube, discard the flowthrough liquid and the Collection Tube.
- Replace the Collection Tube.

Step 5

- Add **450 µl Wash Buffer (P3)** into the reservoir of the 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 Spin Column.

Step 6

- Transfer the Spin Column into a nuclease-free 1.5 ml microcentrifuge tube.
- Add **50 µl Elution Buffer (P4)** into the reservoir of the Spin Column.
- Incubate for 1 min at room temperature.
- Centrifuge 1 min at 8,000 × g.
- The eluate contains purified nucleic acid.

10 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.

Troubleshooting

Kit stored under non-optimal conditions.	Store kit at +18 to +25°C at all times upon arrival.
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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 PolyA/Carrier RNA (PA) after reconstitution and store aliquots at ≤-18°C.
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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.
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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.
Incomplete Proteinase K digestion	Be sure to dissolve the lyophilized Proteinase K completely, as follows: <ol style="list-style-type: none"> 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. 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.

11 Kit Performance

The scope of the validation was to show the performance characteristics of NukEx Pure RNA/DNA and if the method meets the requirements of the intended application, that is to extract DNA and RNA from various biological and environmental samples. During validation, NukEx Pure 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 manufacturer's instructions.

11.1 Sample Material

Table 5: Overview of the samples tested.

Sample	Pathogens detected	Genomic DNA detected
Avian faeces	Influenzaviruses	nd
Buccal swabs	Influenzaviruses, Adenovirus, Respiratory Syncytial Virus, M. tuberculosis	nd
Cerebrospinal fluid	Enteroviruses, Tick-borne encephalitis Virus	nd
Bacterial cultures	E. coli, Streptococci, Legionella, Mycobacteria incl. Mycobacterium tuberculosis complex, Salmonella, Listeria, Campylobacter, Shigella	nd
Bovine blood samples	Bovine Viral Diarrhea Virus (BVD)	nd
Bovine brain samples	Schmallenberg Virus	nd
Bovine faeces	Mycobacterium avium ssp. paratuberculosis	nd
Bovine raw milk	Mycobacterium avium ssp. paratuberculosis, E. coli, Streptococci, Yeast, Pseudomonas	nd
Bovine tissue samples	Coxiella burnetii (Q Fever)	yes
Drinking water	Legionella	nd
Human blood samples	Cytomegalovirus	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	Cytomegalovirus	nd
Human stool samples	Norovirus, Sapovirus, Astrovirus, Rotavirus, Adenovirus, Salmonella, E. coli	nd
Ovine faeces	Mycobacterium avium ssp. paratuberculosis	nd
Tissue culture samples	Varicella Zoster Virus, Cytomegalovirus, Epstein Barr Virus, Enteroviruses, Polioviruses, Herpes Simplex Virus 1+2, Influenzaviruses, Respiratory Syncytial Virus, Rotavirus, Adenovirus, Babesia	nd
Ticks	Tick-borne encephalitis Virus, Borrelia, Ehrlichia, Babesia	yes

*Samples were tested in a forensic lab.

The samples were either field samples, positive for pathogens (e.g. bovine feces 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).

11.2 DNA Extraction

The following table shows an overview of the performance of DNA extraction (genomic, bacterial, viral) using NukEx Pure RNA/DNA (A) in comparison to competitors, indicated by characters in the first row of table 6.

The +/++/+++ indicate the DNA yield and outcome of the subsequently performed real time PCR for the respective pathogens mentioned in table 7 (Stratagene Mx3005P, Roche LightCycler 480II):

- + = Ct range >32
- ++ = Ct range 26 – 32
- +++ = Ct range <26
- na = not applicable
- nd = not done

Table 6: 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 culture	+++	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 6 indicate, that NukEx Pure 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 Pure 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 feces and sputum, and RNA and DNA from ticks, a bead-beating or other mechanical disruption pre-extraction treatment is highly recommended.

11.3 RNA Extraction

The following table shows an overview of the performance of viral RNA extraction using NukEx Pure RNA/DNA (A) in comparison to competitors, indicated by characters in the first row of table 9.

The +/+/+++ indicate the RNA yield and outcome of the subsequently performed real time RT-PCR for the respective pathogens mentioned in table 9 (Stratagene Mx3005P, Roche LightCycler 480II):

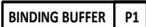
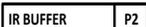
- + = Ct range >32
- ++ = Ct range 26 – 32
- +++ = Ct range <26
- na = not applicable
- nd = not done

Table 9: Comparison of RNA extraction efficiencies.

Sample	A	B	C	D	E	F	G	H	I	K	L	M
Avian faeces	+++	+++	na									
Buccal swabs	+++	+++	na	++								
Liquor	+++	+++	nd	na	na	na	na	na	na	++	na	++
Bovine blood	+++	++	++	na	na	na	na	na	na	++	na	++
Bovine brain	+++	++	na	na	na	na	na	na	++	na	na	na
Bovine faeces	+++	na	++	na								
Bovine raw milk	+++	na	na	na	na	na	na	na	na	na	na	na
Bovine tissue	+++	++	na									
Human urine	+++	na	nd	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 9 indicate, that NukEx Pure 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 highly recommended.

12 Abbreviations and Symbols

DNA	Deoxyribonucleic Acid		Catalog number
RNA	Ribonucleic Acid		Contains sufficient for <n> test
PCR	Polymerase Chain Reaction		Storage at various temperatures
RT	Reverse Transcription		Manufacturer
	Binding Buffer (P1)		Use by YYYY-MM-DD
	Inhibitor Removal Buffer (P2)		Batch code
	Wash Buffer (P3)		Content
	Elution Buffer (P4)		Consult instructions for use
	PolyA/Carrier RNA (PA)		European Conformity
	Spin Columns		<i>In vitro</i> diagnostic medical device
	Collection Tubes		



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

13 Literature

- [1] James H. Jorgensen , Michael A. Pfaller, Karen C. Carroll. Manual of Clinical Microbiology, 11th Edition, 2015.
- [2] Richard L. Hodinka, Benjamin Pinsky. Clinical Virology Manual, 5th Edition, 2016.