Instruction for Use

NukEx Complete RNA/DNA

For extraction of nucleic acids without Proteinase K digest.

REF	**G05020-100**	**G05020-200**
Σ	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Carrier RNA solution has been replaced by PolyA/Carrier RNA lyophilized powder.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

The NukEx Complete RNA/DNA Kit is designed for the extraction of nucleic acids from a wide range of biological 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 (PV1) 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 Complete RNA/DNA G05020-100 is designed for 100 extractions.
NukEx Complete RNA/DNA G05020-200 is designed for 200 extractions.

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

Labelling		**Content**	
		G05020-100	**G05020-200**
PV1	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 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
- 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 Complete 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 Poly A/Carrier RNA solution has to be aliquoted. Aliquots stored at ≤-18°C are stable through date of expiry printed on kit label.
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6 General Information

- 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.
- 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 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 upon request from gerbion.

7 Preparation of Solutions

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

Label	Preparation		Storage and Stability
	G05020-100	G05020-200	
Poly A/ 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 (PV1)	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 (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 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	Milk and mastitis milk samples, see gerbion Cat. No. G07016 Sample Preparation Milk Module.
Tissue	≤ 30 mg	500 µl	Homogenization of tissue in PCR-grade water e.g. with NukEx TS (Cat. No. G06007), centrifugation for 5 min at 8.000 x g, use 200 µl of the supernatant.

Important note: NukEx Complete RNA/DNA cannot be used for blood samples and serum or plasma samples.

9 Extraction of Nucleic Acids

Before starting, prepare a working solution of the Binding Buffer (PV1) supplemented with PolyA/Carrier RNA (PA) 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 (PV1)	500 µl x (N+1)
4 µl PolyA/Carrier RNA (PA)	4 µl x (N+1)

Step 1

- Add **500 µl working solution**, freshly prepared, to a nuclease-free 2.0 ml microcentrifuge tube.
- Add **200 µl sample**
- Mix immediately.
- Incubate for 60 min at 60°C (for milk samples 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

- Pipet entire mixture into the reservoir of the Spin Column.
- Centrifuge 1 min at 8,000 × 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 × 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 Spin 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 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.

Troubleshooting	
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 PolyA/Carrier RNA (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.

11 Abbreviations and Symbols

DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
PCR	Polymerase Chain Reaction
RT	Reverse Transcriptase
BINDING BUFFER PV1	Binding Buffer (PV1)
IR BUFFER P2	Inhibitor Removal Buffer (P2)
WASH BUFFER P3	Wash Buffer (P3)
ELUTION BUFFER P4	Elution Buffer (P4)
POLY A/ CARRIER RNA PA	PolyA/Carrier RNA (PA)
SPIN COLUMNS	Spin Columns
COLLECTION TUBES	Collection Tubes

REF	Catalog number
Σ	Contains sufficient for <n> test
+25°C +18°C	Storage at various temperatures
	Manufacturer
	Use by YYYY-MM-DD
LOT	Batch code
CONT	Content
	Consult instructions for use
CE	European Conformity

12 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.