Instruction for Use

NukEx Complete Mag RNA/DNA

For extraction of nucleic acids without Proteinase K digest.

REF	**G05021-100**	**G05021-200**
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Carrier RNA solution has been replaced by PolyA/Carrier RNA lyophilized powder.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 Mag RNA/DNA Kit is designed for rapid manual and automated extraction of nucleic acids from a wide range of biological samples (e.g. human samples, veterinary samples, insects, food samples, etc.). The kit is designed for use with automated magnetic processors (e.g. KingFisher™ Flex or Duo Prime, Microlab STAR, Freedom EVO). 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 NukEx Complete Mag Binding Buffer. Nucleic acids are bound to the NukEx Magnetic Beads.
b) Bound nucleic acids are washed with NukEx Complete Mag Inhibitor Removal Buffer in order to remove PCR inhibitors from the sample.
c) Bound nucleic acids are washed with NukEx Complete Mag Wash Buffer in order to purify them from salts, proteins and other cellular impurities.
d) Purified nucleic acids are eluted from the NukEx Magnetic Beads with the NukEx Complete Mag Elution Buffer.

Purified nucleic acids can be used directly for downstream applications.

3 Components

NukEx Complete Mag RNA/DNA G05021-100 designed for 100 extractions.
NukEx Complete Mag RNA/DNA G05021-200 designed for 200 extractions.

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, 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 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

Note: consumables not included in the kit are dependent of the mode of sample preparation, e.g. manual extraction or extraction using automated magnetic processors. Therefore, the customer needs to decide, which consumables are necessary for the extraction process.

- Laboratory equipment according to national safety instructions.
- Nuclease-free 1.5 or 2.0 ml 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 Milk Module (gerbion, G07016)
- Thermoblock or laboratory furnace
- Laboratory mixer Vortex or equivalent, or Microplate Shaker
- Vortex Adapter for 24x 1.5 – 2ml tubes or equivalent
- Automated magnetic separator

5 Transport, Storage and Stability

The NukEx Complete Mag RNA/DNA Kit 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.

6 General Information

- 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.
- 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 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 Mag RNA/DNA Solutions.

Label	Preparation		Storage and Stability
	G05021-100	G05021-200	
Poly A/ Carrier RNA (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 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 to each vial, 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 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 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 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 Handling of Magnetic 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 beads are completely resuspended. Shake storage vial well or vortex briefly. Premixing magnetic beads with 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.

10 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 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 PolyA/Carrier RNA (PA)	4 µl x (N+1)

10.1 Protocol for Manual Use

This protocol is for manual use and serves as a guideline for adapting the kit to automated magnetic processors.

Step 1

- Add **20 µl NukEx Magnetic Beads (MB)** to a nuclease-free 2.0 ml microcentrifuge tube. Vortex NukEx Magnetic Beads vigorously before pipetting.
- Add **500 µl working solution,** freshly prepared, to each tube.
- Add **200 µl sample** to each tube.
- Mix immediately.
- Perform incubation 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

- Separate the magnetic beads against the side of the tubes by placing the tubes on a magnetic separator. Wait at least 30 sec 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 µl Inhibitor Removal Buffer (P2)** and resuspend the beads by shaking (optionally mix by pipetting up and down) until the beads are resuspended completely.
- Separate the magnetic beads against the side of the tubes by placing the tubes on the magnetic separator. Wait at least 30 sec 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 town) until the beads are resuspended completely.
- Separate the magnetic beads against the side of the tubes by placing the tubes on the magnetic separator. Wait at least 30 sec 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 5

- Repeat Step 4.

Step 6

- Air-dry the magnetic bead pellet for 5-10 min at room temperature.

Step 7

- Remove the tubes from the magnetic separator.
- Add 100 µl **Elution Buffer (P4)** and resuspend the beads by shaking (optionally mix by pipetting up and down) until the beads are resuspended completely.
- Incubate for 10 min at room temperature.
- Separate the magnetic beads against the side of the tubes by placing the tubes on the magnetic separator. Wait at least 30 sec until all the beads have been attracted to the magnets.
- The supernatant contains purified nucleic acid.
- Transfer the supernatant to fresh nuclease-free tubes.

10.2 Protocol for KingFisher™ Flex Magnetic Particle Processor

Protocols for other automated magnetic particle processors need to be adapted accordingly.

Step 1

- Add **20 µl NukEx Magnetic Beads (MB)** 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 96-well deep-well block.
- Add **450 µl Wash Buffer (P3)** to each well of a second empty 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 KingFisher™ Flex Magnetic Particle Processor use the settings profile shown in Table 5 and Table 6.

Table 5: Reagent Information

Tip plate	Micotiter DW 96 plate	
Lysis/Binding		
Name	*Well volume [µl]*	*Type*
Magnetic Beads	20	Reagent
Working Solution	500*	Reagent
Extraction Control	See Instruction Manual of the respective PCR Kit.	Reagent
Sample	200	Sample
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	100	Reagent

Table 6: Instrument Settings

	Tip 1		96 DW tip comb	
	Pick-Up		Tip plate	
	BInding		Lysis	
			Pause	
		Beginning of step	Precollect	No
			Release beads	Yes
		Mixing / heating	Mixing time, speed	01:00:00, Bottom mix 00:30:00, Bottom mix for milk samples
			Heating during mixing	Yes
			Heating temperature [°C]	60
		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			
			Dry time	00:05:00
			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 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	
Insufficient elution buffer volume	Bead pellet must be covered completely with elution buffer.
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 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 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 and 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. 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 Abbreviations and Symbols

DNA	Deoxyribonucleic Acid	REF	Catalog number
RNA	Ribonucleic Acid	Σ	Contains sufficient for <n> tests
PCR	Polymerase Chain Reaction	+25°C +18°C	Storage at various temperatures
RT	Reverse Transcriptase		Manufacturer
BINDING BUFFER PV1	Binding Buffer (PV1)		Use by YYYY-MM-DD
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/ CARRIER RNA PA	PolyA/Carrier RNA (PA)	CE	European Conformity
MAGNETIC BEADS MB	Magnetic Beads (MB)		

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.