

Instruction for Use

NukEx Mag RNA/DNA

For extraction of nucleic acids using magnetic beads.

REF

G05012-200

G05012-800



200

800



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

IVD



Index

| | | |
|------|---|----|
| 1 | Intended Use..... | 3 |
| 2 | Mode of Action | 3 |
| 3 | Components..... | 3 |
| 4 | Equipment and Reagents to be Supplied by User | 3 |
| 5 | Transport, Storage and Stability | 4 |
| 6 | General Information | 4 |
| 6.1 | Waste Handling | 5 |
| 7 | Preparation of Solutions | 5 |
| 8 | Sample Material..... | 6 |
| 9 | Handling of Magnetic Beads | 6 |
| 10 | Extraction of Nucleic Acids..... | 7 |
| 10.1 | Protocol for Manual Use | 7 |
| 10.2 | Protocol for KingFisher™ Flex Magnetic Particle Processor | 9 |
| 11 | Troubleshooting..... | 12 |
| 12 | Kit Performance | 13 |
| 12.1 | Sample Material | 14 |
| 12.2 | DNA Extraction | 15 |
| 12.3 | RNA Extraction | 17 |
| 13 | Abbreviations and Symbols..... | 18 |

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 automated magnetic processors (e.g. KingFisher™ Flex or Duo Prime, Microlab STAR, Freedom EVO, Biocomma M32, M96). 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

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

Purified nucleic acids can be used directly for downstream applications.

3 Components

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

NukEx Mag RNA/DNA G05012-800 is designed for 800 isolations.

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

| Labelling | | Content | |
|-----------|--------------------------|------------|--------------------------|
| | | G05012-200 | G05012-800 |
| P1 | Binding Buffer | 2 x 30 ml | 2 x 120 ml or 1 x 240 ml |
| PA | PolyA/Carrier RNA | 1 x 4 mg | 4 x 4 mg |
| P2 | Inhibitor Removal Buffer | 2 x 33 ml | 2 x 132 ml or 1 x 264 ml |
| P3 | Wash Buffer | 2 x 20 ml | 4 x 40 ml or 2 x 80 ml |
| P4 | Elution Buffer | 1 x 21 ml | 1 x 84 ml |
| MB | NukEx Magnetic Beads | 4 x 1.0 ml | 1 x 16.0 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

Note: Consumables not included in the kit are dependent of the mode of sample preparation, e.g. manual extraction or extraction using extraction robots. Therefore,

the customer needs to decide, which consumables are necessary for the extraction process.

- Laboratory equipment according to national safety instructions
- Proteinase K (e.g. gerbion G07001 or G07019)
- Nuclease-free 1.5 or 2.0 ml microcentrifuge tubes
- 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. KingFisher96tip comb for DW magnets)
- Tabletop microcentrifuge capable of 13,000 x g centrifugal force
- Absolute ethanol
- 2-propanol
- Thermoblock or laboratory furnace
- Magnetic Particle Processor or magnetic separator

5 Transport, Storage and Stability

NukEx Mag 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 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.
- 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 Mag RNA/DNA Solutions.

| Label | Preparation | | Storage and Stability |
|-------------------------------|---|---|---|
| | G05012-200 | G05012-800 | |
| PolyA/ Carrier RNA (PA) | Dissolve in 1 ml Elution Buffer and prepare aliquots. | Dissolve each vial in 1 ml Elution Buffer and prepare aliquots. | Store at $\leq -18^{\circ}\text{C}$. Stable through date of expiry printed on kit label. |
| Binding Buffer (P1) | Add 26 ml 2-propanol to each vial, mix well. Label and date bottle accordingly. | Filling 2 x 120 ml: Add 104 ml 2-propanol to each vial, mix well. Filling 1 x 240 ml: Add 208 ml 2-propanol to vial, mix well. Label and date bottle accordingly. | Store at $+18$ to $+25^{\circ}\text{C}$. Stable through date of expiry printed on kit label. |
| Inhibitor Removal Buffer (P2) | Add 20 ml absolute ethanol to each vial, mix well. Label and date bottle accordingly. | Filling 2 x 132 ml: Add 80 ml ethanol to each vial, mix well. Filling 1 x 264 ml: Add 160 ml ethanol to 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. | Filling 4 x 40 ml: Add 160 ml ethanol to each vial, mix well. Filling 2 x 80 ml: Add 320 ml 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, briefly spin down sediments. Use 200 µl of supernatant. |
| Swabs | | 500 µl | Add 500 µl PCR-grade water to a dry swab, suspend vigorously and use 200 µl of the suspension. |
| Liquid samples * | 200 µl | 500 µl | |
| Tissues | ≤ 30 mg | 500 µl | Homogenize tissue in 500 µl PCR-grade water e.g. with NukEx TS (gerbion, Cat. No. G06007) using NukEx Pestle (gerbion, Cat. No. G06006) or 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 | Pellet up to 2 x 10 ⁶ cells. Resuspend pellet in 200 µl PCR-grade water. Homogenization e.g. with NukEx TS (gerbion, Cat. G06007) using NukEx Pestle (gerbion, Cat. 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. |

*EDTA-blood, serum, amniotic fluid, CSF, urine, water, milk etc.

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 (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.

Samples containing precipitates must be centrifuged before purification!

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

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) |

10.1 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 **20 μl Magnetic Beads (MB)** to a nuclease-free 2.0 ml microcentrifuge tube. Vortex Magnetic Beads vigorously before pipetting.
- Add **550 μl working solution**, freshly prepared, to each tube.
- Add **200 μl sample** to each tube.
- Mix immediately.
- Perform incubation for 10 min at 60°C .
- Following the incubation, centrifuge briefly to collect any sample from the lysis tube lid.

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 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 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 (optional 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 Magnetic Beads (MB)** to each well of an empty 96 deep-well block. Vortex Magnetic Beads vigorously before pipetting.
- Add **550 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 the KingFisher™ Flex Magnetic Particle Processors use the settings profile shown in table 5 and table 6.

Table 5: Reagent Information

| | | |
|--------------------------|---|-------------|
| Tip plate | Microtiter DW 96 plate | |
| Lysis/Binding | | |
| <i>Name</i> | <i>Well volume [μl]</i> | <i>Type</i> |
| Magnet Beads | 20 | Reagent |
| Working Solution | 550 | Reagent |
| Extraction Control | See Instruction Manual of the respective PCR Kit. | Reagent |
| Sample | 200 | Sample |
| Inhibitor Removal Buffer | Inhibitor Removal | |
| <i>Name</i> | <i>Well volume [μl]</i> | <i>Type</i> |
| Inhibitor Removal | 500 | Reagent |
| 1st Wash Buffer | Inhibitor Removal | |
| <i>Name</i> | <i>Well volume [μl]</i> | <i>Type</i> |
| Wash Buffer | 450 | Reagent |
| 2nd Wash Buffer | 2nd Wash Buffer | |
| <i>Name</i> | <i>Well volume [μl]</i> | <i>Type</i> |
| Wash Buffer | 450 | Reagent |
| Elution | Elution | |
| <i>Name</i> | <i>Well volume [μl]</i> | <i>Type</i> |
| 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 | 00:10:00, Bottom mix |
| | | Heating during mixing | Yes |
| | | Heating temperature [°C] | 60 |
| | End of step | Postmix | No |
| | | Collect count | 4 |
| | | Collect time [s] | 3 |
|  | Inhibitor Buffer | Removal | 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, 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 extraction, please do not hesitate to contact our scientists on info@gerbion.com.

| Low nucleic acid yield | |
|---|---|
| Sample not sufficiently lysed | Supplement the working solution (Binding Buffer + PolyA/Carrier RNA (PA) 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: <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 rehydrated enzyme, mark each aliquot with the date of reconstitution, and store at $\leq -18^{\circ}\text{C}$. Rehydrated Proteinase K is stable for 12 months when stored properly. |
| 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, 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 PolyA/Carrier RNA (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 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 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 the intended application, that is to extract DNA and RNA from various biological and environmental samples. During validation, NukEx Mag RNA/DNA was tested against other commercial extraction kits using standardized samples. Kits B to M mentioned in Tables 8 and 9 represent recommended kits of the manufacturers for the respective sample materials. 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.

12.1 Sample Material

Table 7: 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 these materials in infected subjects (e.g. urine spiked with Cytomegalovirus, buccal swabs spiked with Influenzaviruses).

12.2 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 table 8.

The +/++/+++ indicate the DNA yield and outcome of the subsequently performed real time PCR for the respective pathogens mentioned in Table 8 (Stratagene Mx3005P, Roche LightCycler 480II):

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

Table 8: 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 feces | +++ | 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 8 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 feces and sputum, and RNA and DNA from ticks, a bead-beating or other mechanical disruption pre-extraction treatment is also highly recommended.

12.3 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 for the respective pathogens mentioned in Table 9 (Stratagene Mx3005P, Roche LightCycler 480II):







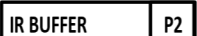










- + = Ct range >32
- ++ = Ct range 26 – 32
- +++ = Ct range <26
- na = not applicable
- nd = not done

Table 9: Comparison of RNA extraction efficiencies

| Sample | A | B | C | D | E | F | G | H | I | K | L | M |
|----------------------|-----|-----|----|----|----|----|----|----|----|----|----|----|
| Avian faeces | +++ | +++ | na | 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 9 indicate, 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 highly recommended.

13 Abbreviations and Symbols

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



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

14 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.