MIKROGEN
DIAGNOSTIK

Instruction for Use

*alpha*Clean Mag RNA/DNA

For extraction of nucleic acids using magnetic beads.

REF	831001	831002
Σ	100	200
+18°C +25°C		

Index

1 Intended Use

The *alpha*Clean 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). 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 Principle of the Test

a) Samples are lysed by incubation in Working Solution (Binding Buffer (P1) substituted with 2-propanol). Nucleic acids are bound to the Magnetic Beads.
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 Magnetic Beads with Elution Buffer (P4).

Purified nucleic acids can be used directly for downstream applications.

3 Package Contents

*alpha*Clean Mag RNA/DNA cat. no. 831001 is designed for 100 isolations.
*alpha*Clean Mag RNA/DNA cat. no. 831002 is designed for 200 isolations

Table 1: Components of the *alpha*Clean Mag RNA/DNA isolation kits.

Labelling		**Content**	
		831001	**831002**
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 10.5 ml	1 x 21 ml
MB	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 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, Mikrogen Cat. No. 831007, 831025
- Nuclease-free 1.5 or 2.0 ml microcentrifuge 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
- 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

*alpha*Clean 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 *alpha*Clean 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.
- 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 Mikrogen upon request.

7 Preparation of Solutions

Table 2: Preparation of *alpha*Clean Mag RNA/DNA Solutions.

Label	Reconstitution/ Preparation		Storage and Stability
	831001	831002	
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 aliquots 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: 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 with e.g. alphaClean TS (831016, 831017, 831018) using 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 with e.g. alphaClean TS (831016, 831017, 831018) using 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.

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 reconstituted 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 ≤-18°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 [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 5 sec at max. speed 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 the 96-well deep-well block.
- Add **450 µl Wash Buffer (P3)** to each well of a second empty the 96-well deep-well block.

Step 2 - 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	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	1st Wash Buffer	
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	
	Working Solution with Magnetic Beads	Lysis/Binding	
		Pause	
	Beginning of step	Precollect	No
		Release beads	Yes
	Mixing / heating	Mixing time, speed	0:10:00, Bottom mix
		Heating during mixing	Yes
		Heating temperature [°C]	60
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	3
	Inhibitor 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, 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 mikrogen@mikrogen.de.

Low nucleic acid yield	
Sample not sufficiently lysed	Supplement the working solution (Binding Buffer + PolyA/Carrier RNA 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: 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 ≤-18°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 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.

12 Kit Performance

The scope of the validation was to show the performance characteristics *alpha*Clean 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, *alpha*Clean 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 faeces 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 Tick-borne encephalitis Virus, 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).

12.2 DNA Extraction

The following table shows an overview of the performance of DNA extraction (genomic, bacterial, viral) using alphaClean Mag RNA/DNA (A) in comparison to competitors, indicated by characters in the first row of the table.
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 8 indicate that *alpha*Clean 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 *alpha*Clean 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 *alpha*Clean 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 8 (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 *alpha*Clean 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 also highly recommended.

13 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	Limit of temperature
RT	Reverse Transcription		Manufacturer
BINDING BUFFER P1	Binding Buffer (P1)		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
PolyA/Carrier RNA PA	PolyA/Carrier RNA (PA)		
MAGNETIC BEADS MB	Magnetic Beads		

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.

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