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Manual

MutaCLEAN®
Universal RNA/DNA
extraction kit

For column based RNA/DNA extractions from different samples

Valid from 2022-05-23

REF **KG1038**

Σ 200

25 °C
18 °C

IVD CE

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1 INTENDED USE

MutaCLEAN® Universal RNA/DNA is a column-based extraction kit for nucleic acids (RNA and DNA) which may be used e.g. for subsequent real time PCR analysis. The kit is an accessory for an in vitro diagnostic device. Any in vitro diagnostic use of this sample preparation procedure in combination with a downstream nucleic acid test must be evaluated for the parameter in question. The nucleic acids can be extracted from clinical or environmental samples incl. food samples. The kit is intended to be used by professional users in a laboratory environment. The extraction is performed manually.

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

MutaCLEAN® Universal RNA/DNA KG1038 is designed for 200 isolations

Table 1: Components of the MutaCLEAN® Universal RNA/DNA isolation kit.

Labelling		Content
		KG1038
P1	Binding buffer	2 x 30 ml add 26 ml 2-propanol, each
PA	Poly A/carrier RNA	4 mg
P2	Inhibitor removal buffer	2 x 33 ml add 20 ml absolute ethanol each
P3	Wash buffer	2 x 20 ml add 80 ml absolute ethanol each
P4	Elution buffer	1 x 12 ml

Labelling		Content
		KG1038
	Spin columns	200 pieces
	Collection tubes	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
- Sterile pipet tips with filter
- Nuclease-free collection tubes
- Nuclease-free 1.5 ml or 2.0 ml microcentrifuge tubes
- Tabletop microcentrifuge capable of 13 000 *g* centrifugal force
- Absolute ethanol
- 2-propanol
- Thermoblock or laboratory furnace

5 TRANSPORT, STORAGE AND STABILITY

MutaCLEAN® Universal 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 until the expiry date printed on the kit label.

6 GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- Read the Instruction for Use carefully before using the product.
- This instruction for use replaces the version dated 2020-06-16.
- This product has been produced and placed on the market in accordance with the regulation (EU) 2017/746 (IVDR).

- All serious incidents occurring in connection with the product must be reported to Immundiagnostik AG and (within the Union market) to the competent reporting authority of the respective member state.
- MutaCLEAN® Universal 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 upon request.

7 PREPARATION OF SOLUTIONS

Table 2: Preparation of MutaCLEAN® Universal RNA/DNA Solutions.

Label	Preparation	Storage and Stability
	KG1038	
Poly A/carrier RNA (PA)	Dissolve in 1.0 ml elution buffer and prepare 50 µl aliquots.	Store aliquots at ≤ -18 °C, stable through the 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.	Store at 18 to 25 °C. Stable through the 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.	Store at 18 to 25 °C. Stable through the date of expiry printed on kit label.
Wash buffer (P3)	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, faeces	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.

Sample Material	Volume / Amount	Volume binding buffer	Pre-treatment of the Sample
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	Homogenisation of tissue in 500 µl PCR-grade water e. g. with tissue homogeniser (e.g. Precellys, Bertin Instruments). Spin down for 1 min at 8 000 *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. Homogenisation e.g. with tissue homogeniser (e.g. Precellys, Bertin Instruments). Spin down for 1 min. at 8 000 *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 ≤-18 °C for later analysis.

9 PROCEDURE

Before starting, prepare a working solution of the Binding Buffer (P1) supplemented with reconstituted poly A/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 poly A/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 s 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 s 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 s 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@immundiagnostik.com.

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 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.

Incomplete Proteinase K digestion

Be sure to dissolve the lyophilised proteinase K completely as follows:

a) Pipet the appropriate volume of PCR grade water to the 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).

b) Close vial and invert until all the lyophilisate (including any stuck to the lid) is completely dissolved.

c) 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 MutaCLEAN® Universal 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, MutaCLEAN® Universal RNA/DNA was tested against other commercial extraction kits using standardised 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	Influenza viruses	nd
Buccal swabs	Influenza viruses, Adenovirus, RSV, *M. tuberculosis*	nd
Cerebrospinal fluid	Enteroviruses, TBEV	nd
Bacterial cultures	*E. coli*, *Streptococci*, *Legionella*, *Mycobacteria* incl. MTB, *Salmonella*, *Listeria*, *Campylobacter*, *Shigella*	nd
Bovine blood samples	Bovine viral diarrhea (BVD) virus	nd
Bovine brain samples	Schmallenberg virus	nd
Bovine faeces	*Mycobacterium avium ssp. paratuberculosis*	nd

Sample	Pathogens detected	Genomic DNA detected
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, HSV 1+2, influenza viruses, RSV, rotavirus, adenovirus, *Babesia*	nd
Ticks	TBEV, *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 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 influenza viruses.

11.2 DNA extraction

The following table shows an overview of the performance of DNA extraction (genomic, bacterial, viral) using MutaCLEAN® Universal 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 6 (Stratagene Mx3005P, Roche LightCycler 480II):

+ = C_t range > 32
++ = C_t range 26–32
+++ = C_t 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 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 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

Sample	A	B	C	D	E	F	G	H	I	K	L	M
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 MutaCLEAN® Universal 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 MutaCLEAN® Universal 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 faeces 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 MutaCLEAN® Universal RNA/DNA (A) in comparison to competitors, indicated by characters in the first row of table 7.

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

+ = C_t range > 32

++ = C_t range 26–32

+++ = C_t range < 26

na = not applicable

nd = not done

Table 7: 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	++
Liquor	+++	+++	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 7 indicate, that MutaCLEAN® Universal 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	Desoxyribonucleic acid	REF / →REF	Catalog number
RNA	Ribonucleid acid	Σ	Contains sufficient for <n> test
PCR	Polymerase chain reaction		Limit of temperature
RT	Reverse transcription		Manufacturer
CMV	Cytomegalovirus		Use by YYYY-MM-DD

MAP	*Mycobacterium avium ssp paratuberculosis*	LOT	Batch code
VZV	Varicella zoster virus	CONT	Content
EBV	Epstein barr virus		Consult instructions for use
HSV	Herpes simplex virus	IVD	*In vitro* diagnostic medical device
RSV	Respiratory syncytial virus	CE	European Conformity
SBV	Schmallenberg virus	COLLECTION TUBES	Collection tubes
BINDING BUFFER P1	Binding buffer (P1)	ELUTION BUFFER P4	Elution buffer (P4)
IR BUFFER P2	Inhibitor removal buffer (P2)	POLY A/CARRIER RNA PA	Poly A (PA)
WASH BUFFER P3	Wash buffer (P3)	SPIN COLUMNS	Spin columns
UDI	Unique Device Identification		Attention
	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.