Instruction for Use

NukEx Pure RNA/DNA

For general laboratory use.
For *in vitro* use only.

For isolation of nucleic acids using columns.

REF	G05004-50	G05004-200
Σ	50	200

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1 Intended Use

NukEx Pure RNA/DNA Kit is designed for rapid manual purification of nucleic acids from a wide range of samples (e.g. human samples, veterinary samples, insects, food samples, etc.). The purified RNA / DNA can be used directly as template for RT-PCR, PCR, or any kind of enzymatic reactions.

2 Mode of Action

For the analysis of nucleic acids by PCR or RT-PCR, the isolation of the analyte from various sample materials is required. To this end, the sample is lysed by incubation in a proprietary lysis buffer (Binding Buffer (P1), supplemented with Proteinase K).

In the presence of a chaotropic salt, nucleic acids bind specifically to the surface of the glass fibres within the NukEx Spin Columns. Specifically bound to the glass fibres, the nucleic acids can be purified from salts, proteins and other components found in the sample using Inhibitor Removal Buffer (P2) and Wash Buffer (P3). Residual ethanol from previous wash steps is removed by air drying. Finally, highly pure RNA / DNA is eluted with low-salt Elution Buffer (P4). Purified RNA / DNA can directly be used for downstream applications.

1. Samples are lysed by incubation in NukEx Binding Buffer (P1). Nucleic acids are bound to the glass fibres within the NukEx Spin Columns.
2. Bound nucleic acids are washed with NukEx Inhibitor Removal Buffer (P2) in order to remove PCR inhibitors from the sample such as >100 U/ml heparin.
3. Bound nucleic acids are washed with NukEx Wash Buffer (P3) in order to purify them from salts, proteins and other cellular impurities.
4. Purified nucleic acids are eluted from the NukEx Magnetic Beads with the NukEx Elution Buffer (P4).

3 Components

NukEx Pure RNA/DNA G05004-50 is designed for 50 isolations.
NukEx Pure RNA/DNA G05004-200 is designed for 200 isolations

Table 1: Components of the NukEx Pure RNA/DNA isolation kits.

Labelling		Content G05004-50	G05004-200
P1	Binding Buffer	1 x 21 ml add 9 ml 2-propanol	2 x 40 ml add 17 ml 2-propanol each
PA	Poly A	1 mg	4 mg
P2	Inhibitor Removal Buffer	1 x 16.5 ml add 10 ml absolute ethanol	2 x 33 ml add 20 ml absolute ethanol each
P3	Wash Buffer	1 x 10 ml add 40 ml absolute ethanol	2 x 20 ml add 80 ml absolute ethanol each
P4	Elution Buffer	1 x 4.5 ml	1 x 12 ml
	NukEx Spin Columns	50 pieces	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.
- PCR grade water
- Sterile pipet tips with filter
- Nuclease-free Collection Tubes (gerbion G06008)
- Nuclease-free 1.5 ml or 2.0 ml microcentrifuge tube
- Tabletop microcentrifuge capable of 13,000 x g centrifugal force
- Absolute ethanol
- 2-propanol
- Proteinase K (e.g. gerbion G07001)
- Thermoblock or laboratory furnace

5 Transport, Storage and Stability

NukEx Pure RNA/DNA Kit components are shipped at ambient temperature. NukEx Pure RNA/DNA Kit components 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 for 12 months.

6 General Information

6.1 Important Notes

- The NukEx Pure 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.

6.2 Precautions

- 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 use bleach for waste treatment!
- 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.
- Never store or use the buffers near human or animal food.
- Always wear gloves and follow standard safety precautions when handling these buffers.

6.3 Handling Requirements

- Exercise the normal precautions required for handling of all laboratory reagents.
- 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.
- Use only calibrated pipettes.

6.4 Laboratory Procedures

- All sourced material and all resulting waste should be considered potentially infectious. Thoroughly clean and disinfect all work surfaces with disinfectants recommended by the local authorities.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection when handling specimens and kit reagents.
- Avoid microbial and nuclease contamination of reagents when removing aliquots from reagent bottles.
- The use of sterile disposable pipettes is recommended.
- Wash hands thoroughly after handling samples and test reagents.

6.5 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 Pure RNA/DNA Solutions.

Label	Reconstitution/ Preparation		Storage and Stability	Purpose
	G05004-50	G05004-200		
Poly A (PA)	Dissolve in 0.25 ml Elution Buffer and prepare 50 µl aliquots.	Dissolve in 1.0 ml Elution Buffer and prepare 50 µl aliquots.	Store aliquots at ≤-18°C, stable for 12 months.	Supplement of the Binding Buffer for isolation of nucleic acids
Binding Buffer (P1)	Add 9 ml 2-propanol to Binding Buffer and mix well. Label and date bottle accordingly after adding 2-propanol.	Add 17 ml 2-propanol to each vial Binding Buffer and mix well. Label and date bottle accordingly after adding 2-propanol.	Store at +18 to +25°C. Stable through the date of expiry printed on the kit label.	Sample lysis
Inhibitor Removal Buffer (P2)	Add 10 ml absolute ethanol to Inhibitor Removal Buffer and mix well. Label and date bottle accordingly after adding ethanol.	Add 20 ml absolute ethanol to each vial Inhibitor Removal Buffer and mix well. Label and date bottle accordingly after adding ethanol.	Store at +18 to +25°C. Stable through the date of expiry printed on the kit label.	Removal of PCR inhibitors from the bound nucleic acid
Wash Buffer (P3)	Add 40 ml absolute ethanol to each vial Wash Buffer and mix well. Label and date bottle accordingly after adding ethanol.	Add 80 ml absolute ethanol to each vial Wash Buffer and mix well. Label and date bottle accordingly after adding ethanol.	Store at +18 to +25°C. Stable through the date of expiry printed on the kit label.	Removal of salts, proteins and other residual impurities from the bound nucleic acid

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) and pre-treatment of the sample 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 sterile $_dH_2O$. Vortex and briefly spin down sediments. Use 200 µl of supernatants.
Swabs		500 µl	
Liquid samples*	200 µl	500 µl	
Animal/ human tissues	≤ 30 mg	500 µl	Homogenization of tissue in Binding Buffer e.g. with NukEx TS (gerbion G06007)
animal / human cells	≤2 x 10^6	500 µl	Harvest and pellet up to 2 x 10^6 cells. Resuspend pellet in Binding Buffer.

*Liquid samples such as EDTA-blood, serum, amniotic fluid, CSF, urine, water, milk etc.

9 Preparation Time

The preparation time needed is always dependent on the number of samples to be prepared.

Table 4: Preparation times for the isolation of nucleic acids with NukEx Pure RNA/DNA kit.

	DNA / RNA
Total time	approx. 40 minutes
Hands-on time	less than 10 minutes

10 Procedure

Procedures below are for preparing nucleic acids from 200 µl sample volume. If solid sample matrices are to be used please refer to Table 3 for appropriate buffer volumes. For information on the extraction from other sample matrices please contact our scientists on info@gerbion.com.

Samples containing precipitates must be centrifuged before purification!

Store eluted nucleic acid at ≤-18°C for later analysis.

Before starting, prepare a working solution of the Binding Buffer (P1) supplemented with reconstituted Poly A (PA) and Proteinase K for at least one sample (N) more than required in order to compensate for frothing of the buffer.

Table 5: 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 (PA)	4 µl x (N+1)
50 µl Proteinase K [20 mg/ml]	*50 µl x (N+1)*

Pre-warming (56-60°C) of Elution Buffer (P4) can increase the yield of nucleic acids.

Step 1

- Add **200 µl sample** to a nuclease-free 2.0 ml microcentrifuge tube.
- Add **550 µl working solution**, freshly prepared.
- Mix immediately.
- Incubate for 10 min at 56-60°C.

Step 2

- Pipet entire mixture into the reservoir of the NukEx Spin Column.
- Centrifuge 1 min at 8,000 × g.
- Remove the NukEx 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 NukEx Spin Column.
- Centrifuge 30 s at 8,000 × g.
- Remove the NukEx 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 NukEx Spin Column.
- Centrifuge 30 s at 8,000 × g.
- Remove the NukEx Spin Column from the Collection Tube, discard the flowthrough liquid and the Collection Tube.
- Replace the Collection Tube.
- *Optional*: Add **450 µl Wash Buffer (P3)** into the reservoir of the NukEx 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 column.

Step 5

- Transfer the NukEx Spin Column into a nuclease-free 1.5 ml microcentrifuge tube.
- Add **20-50 µl (*optional: pre-warmed [60°C]*) Elution Buffer (P4)** into the reservoir of the NukEx Spin Column.
- *Optional: Incubate for 1 min at room temperature.*
- Centrifuge 1 min at 8,000 × g.
- The eluate contains purified nucleic acid.

* For complex sample materials, such as whole blood or faeces, an additional wash step with Wash Buffer (P3) can increase purity of the eluated nucleic acid.

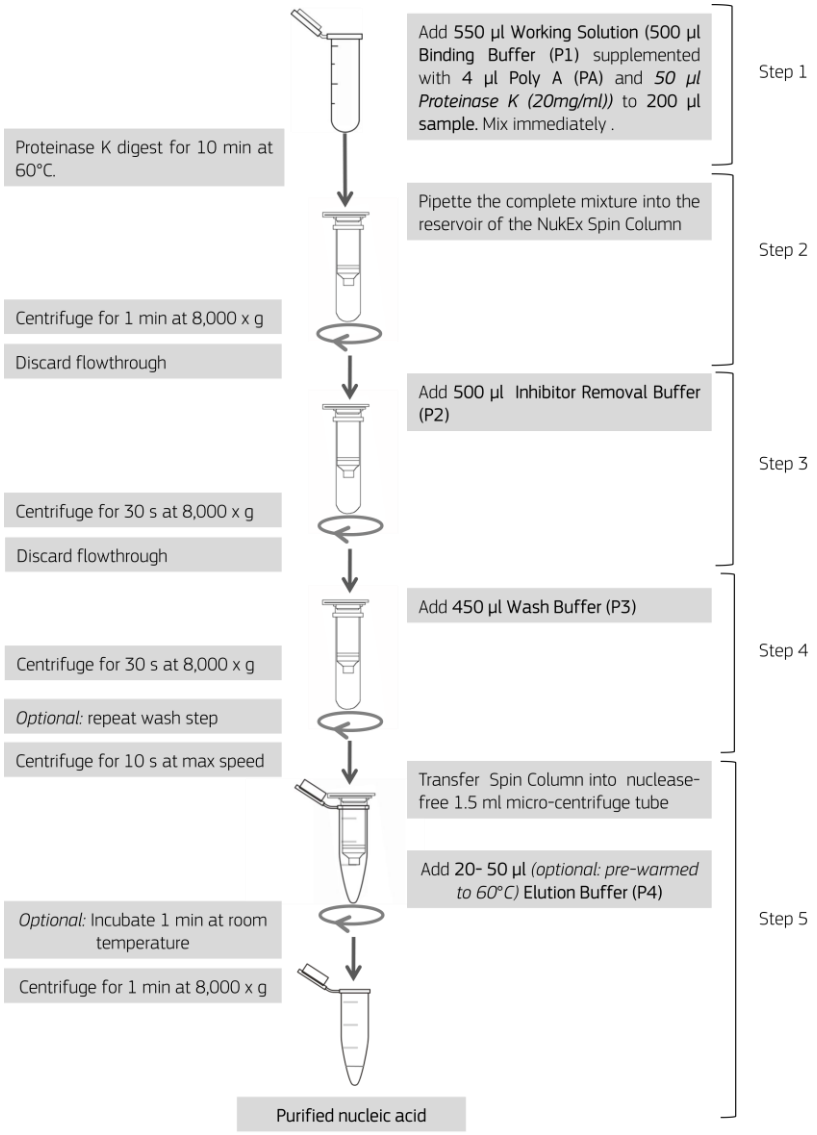

Figure 1: Protocol for the isolation of nucleic acids.

11 Assay Validation

Extraction Control

Use the Extraction Control included in real time (RT-) PCR Kits for downstream processing. E.g. add 5 µl of the Extraction Control per reaction directly to the Binding Buffer and co-elute with the nucleic acid of the sample. The Ct value of the Extraction Control in the subsequent real time (RT-) PCR needs to meet the validation criteria of the respective real time (RT-) PCR Kit.

12 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.

Low nucleic acid yield or purity	
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 Poly A (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.
Sample not sufficiently lysed	Some sample materials - such as whole blood, tissue, or buccal swabs- require addition of Proteinase K in order to sufficiently release nucleic acid from cells. Supplement the working solution (=Binding Buffer + Poly A) 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. 5 ml PCR grade water to 100 mg Proteinase K). 2. Re-close vial and invert until all the lyophilisate (including any stuck to the lid) is completely dissolved. 3. 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.

13 Kit Performance

The scope of the validation was to show that the performance characteristics NukEx Pure RNA/DNA and if the method meets the requirements of their intended application, that is to extract DNA and RNA from clinical and environmental samples.

During validation, NukEx Pure RNA/DNA was tested against other commercial extraction kits using standardized 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.

13.1 Sample Material

Table 6: Overview of the samples tested.

Sample	Pathogens detected	Genomic DNA detected
Avian faeces	Influenzaviruses	nd
Buccal swabs	Influenzaviruses, Adenovirus, RSV	nd
Cerebrospinal fluid	Enteroviruses, TBEV	nd
Bacterial cultures	E. coli, Streptococci, Legionella, Mycobacteria incl. MTB, Salmonella, Listeria, Campylobacter, Shigella	nd
Bovine blood samples	BVDV	nd
Bovine brain samples	SBV	nd
Bovine faeces	MAP	nd
Bovine raw milk	MAP	nd
Bovine tissue samples	Coxiella burnetii (Q Fever)	yes
Drinking water	Legionella	nd
Human blood samples	CMV	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	CMV	nd
Human stool samples	Norovirus, Sapovirus, Astrovirus, Rotavirus, Adenovirus, Salmonella, E. coli	nd
Ovine faeces	MAP	nd
Tissue culture samples	VZV, CMV, EBV, Enteroviruses, Polioviruses, HSV 1+2, Influenzaviruses 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 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 this materials in infected subjects (e.g. urine spiked with Cytomegalovirus, buccal swabs spiked with Influenzaviruses, see table 6).

13.2 DNA Extraction

The following table shows an overview of the performance of DNA extraction (genomic, bacterial, viral) using NukEx Pure 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.
na = not applicable
nd = not done

Table 7: 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 sample	+++	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 sampl	+++	na	na	++	na	na	na	++	na	na	na	na
Drinking water	+++	na	+++	+++	na	+++	na	na	na	na	nd	na
Human blood sampl	+++	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 samp	+++	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 7 indicate, that NukEx Pure 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 Pure 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 also highly recommended.

13.3 RNA Extraction

The following table shows an overview of the performance of viral RNA extraction using NukEx Pure 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.
na = not applicable
nd = not done

Table 8: 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 8 indicates, that NukEx Pure 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.

14 Abbreviations and Symbols

DNA	Desoxyribonucleic Acid	REF	Catalog number
RNA	Ribonucleid Acid	Σ	Contains sufficient for <n> test
PCR	Polymerase Chain Reaction	+25°C +18°C	Limit of temperature
RT	Reverse Transcription		Manufacturer
CMV	Cytomegalovirus		Use by YYYY-MM
MAP	Mycobacterium avium ssp paratubeculosis	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	SBV	Schmallenberg Virus
BINDING BUFFER P1	Binding Buffer (P1)	ELUTION BUFFER P4	Elution Buffer (P4)
IR BUFFER P2	Inhibitor Removal Buffer (P2)	POLY A PA	Poly A (PA)
WASH BUFFER P3	Wash Buffer (P3)	SPIN COLUMNS	Spin Columns

	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

15 Literature

[1] Sambrook, J. and Russell, D.W.: Molecular Cloning, 2001.