VIASURE

RNA-DNA

Extraction Kit

VIASURE

Instruction for VIASURE RNA-DNA Extraction Kit

The **VIASURE RNA-DNA Extraction Kit** provides a fast and easy method for isolation and purification of high pure total nucleic acid. Total genomic, bacterial DNA and viral DNA/RNA can be purified from 200 µl of fresh or frozen cell cultures, tissues, plasma, serum, urine, cell free body fluids as well as rinsed liquid from swabs, pretreated sputum, bronchoalveolar lavage (BAL), breast milk and supernatant from stool suspension or whole blood (100 µl), and additionally, only for veterinary applications from allantoic fluid or rinse liquid from cloacal or tracheal swabs.

Due to the high purity, the isolated DNA/RNA is ready to use for broad panel downstream applications or can be stored at -20°C/-80°C for subsequent use.

CE IVD Compliance with EU Directive 98/79/EC on *in vitro* medical devices.

Not for *in-vitro* diagnostic use in countries where the EU Directive 98/79/EC on *in vitro* medical devices is not recognized.

Contents

1. Kit contents

	50 Preparations
Lysis Buffer	15 ml
Proteinase K	for 1.1 ml working solution
Carrier RNA	for 1.2 ml working solution
RNase Free Water	2 x 2 ml
Binding Buffer (fill with 99.7% Isopropanol)	empty bottle (final volume 15 ml)
Wash Buffer I	30 ml (final Volume 50 ml)*
Wash Buffer II	2 x 18 ml (final Volume 2 x 60 ml)*
Elution Buffer	30 ml
Mini Spin Column Set	50
RTA Collection Tubes	2 x 50
1.5 ml Collection Tubes	50
2.0 ml Collection Tubes	50
Instructions for use	1
Initial steps	Add 1.1 ml **RNase free Water** to **Proteinase K** vial for resuspending the lyophillized. Immediately, mix thoroughly by shaking until completely dissolving and store at -20°C. Resuspend lyophillized **Carrier RNA** by addition of 1.2 ml **RNase free Water** to the vial and mix thoroughly by shaking until completely dissolving. Fill the empty bottle **Binding Buffer** with 15 ml 99.7% **Isopropanol**. * **Wash Buffer I** and **Wash Buffer II** are supplied as concentrates. Add Isopropanol or Ethanol according to the bottle label before use to obtain a working solution. Before using the first time, add 20 ml of 99.7% Isopropanol to the bottle **Wash Buffer I.** Mix thoroughly and keep it always firmly closed to avoid alcohol evaporation. Make a mark on the bottle label to indicate that Isopropanol has been added. Before using the first time, add 42 ml of 99.8% Ethanol to the bottle **Wash Buffer II**. Mix thoroughly and keep it always firmly closed to avoid ethanol evaporation.Make a mark on the bottle label to indicate that Ethanol has been added.

2. Symbols

Lot number

Catalogue number


Date of manufacture


Expiry date


Consult operating instructions


Temperature limitation


Do not reuse


Manufacturer

3. Storage

All buffers and kit contents of the **VIASURE RNA-DNA Extraction Kit** should be stored at room temperature (RT, 15-30ºC) until expiry date which is indicated in the exterior label of the product.

Proteinase K: Dissolved Proteinase K must be stored at -20°C until the expiry date of the kit. Dividing the Proteinase K into aliquots and storage at -20°C is recommended.

Carrier RNA: Lyophilized Carrier RNA can be stored at 2 - 8°C or at RT, but the recommendation for long time storage is -20°C. Dissolved Carrier RNA must be stored at -80°C, but repeated freezing and thawing will degrade the Carrier RNA and reduce the functionality of the kit. Therefore, dividing Carrier RNA into aliquots and storage at -80°C is recommended.

Wash Buffers: Wash Buffers charged with isopropanol or ethanol should be stored at RT appropriately sealed until the expiry date of the kit. If any precipitates are visible within the provided solutions, solve these precipitates by careful warming up to 30°C.

4. Quality control and product warranty

CerTest guarantees the correct function of the **VIASURE RNA-DNA Extraction Kit** for the applications in the manner described in this manual. The purchaser must determine the suitability of the product for its particular use. Should any Product fail to perform satisfactorily due to any reason other than misuse, CerTest will check the lot and if there is a problem, CerTest will replace it free of charge.

CerTest reserves the right to change, alter, or modify any product to enhance its performance and design at any time.

In accordance with CERTEST's ISO EN 13485 certified Quality Management System the performance of all components of the **VIASURE RNA-DNA Extraction Kit** have been tested separately against predetermined specifications routinely on lot-to-lot to ensure consistent product quality.

If you have any questions or problems regarding any aspects of **VIASURE RNA-DNA Extraction Kit** or other CerTest products, please do not hesitate to contact us. A copy of CerTest's terms and conditions can be obtained on request or are presented at the CerTest webpage (www.certest.es)

For technical support or further information please contact: +34 976520354 or certest@certest.es or contact directly with your local distributor.

5. Intended use

The **VIASURE RNA-DNA Extraction Kit** is designed for rapid extraction and purification of nucleic acids (genomic and bacteria DNA and viral DNA/RNA) from 200 µl of variety of sample sources (for blood sample only 100 µl) using the Mini Spin Column system with capped spin column.

The nucleic acid isolation protocol is suitable for routinely preparation of DNA/RNA from fresh material or material that has been immediately frozen and stored at -20ºC or -80ºC. For reproducible and high yields an appropriate sample storage is essential (see section 14 “Sampling and storage of the starting material”).

The **VIASURE RNA-DNA Extraction Kit** is the ideal tool for reliable and fast simultaneous isolation of nucleic acids (genomic and bacteria DNA and viral DNA/RNA) from fresh or frozen human or mammalian tissue, blood, serum, plasma, swabs, cerebrospinal fluid, cell culture supernatants, urine, supernatant from stool suspension and other cell free body fluids.

The kit can also be used for the isolation of high quality genomic, bacterial and viral DNA as well as viral RNA from the same kind of samples but coming from animals. The amount of blood depends on the kind of animals.

Blood samples have to be stabilized with EDTA or citrate, not heparin.

The isolation protocols as well as all buffers are optimized to provide high yield and purity of the extracted nucleic acids. The procedure requires minimal interaction by the user, allowing safe handling of potentially infectious samples.

THE PRODUCT IS INDENTED FOR USE BY PROFESSIONAL USERS ONLY, SUCH AS LABORATORY OR HEALTH PROFESSIONALS AND TECHNICIANS, TRAINED IN MOLECULAR BIOLOGICAL TECHNIQUES.

It is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of DNA followed by signal detection or amplification. Any diagnostic results generated by using the sample preparation procedure in conjunction with any downstream diagnostic assay should be interpreted with regard to other clinical or laboratory findings. To minimize irregularities in diagnostic results, adequate controls for downstream applications should be used.

The kit is in compliance with EU Directive 98/79/EC on *in vitro* medical devices. But it is not for *in vitro* diagnostic use in countries where the EU Directive 98/79/EC on *in vitro* medical devices is not recognized.

6. Product use limitations

The product has not been evaluated neither validated for the isolation of eukaryotic total RNA from any kind of sample. The isolation of RNA from sample sources like fungi was neither tested nor validated.

Differing the starting material or flow trace may lead to inoperability. Therefore, neither a warranty nor a guarantee in this case will be given, implied or expressed.

The user is responsible to validate the performance of the CerTest product for any particular use.

CerTest does not provide validations of performance characteristics of the product with respect to specific applications.

CerTest products may be used in clinical diagnostic laboratory systems conditioned upon the complete diagnostic system of the laboratory. The laboratory must be validated pursuant to CLIA’ 88 regulations in the U.S. or equivalents in other countries.

All products sold by CerTest are subject to extensive quality control procedures (according ISO EN 13485) and are warranted to perform as described herein. Any problems, incidents or defects shall be reported to CerTest immediately upon detection thereof.

The chemicals and the plastic parts are for laboratory use only. They must be stored in the laboratory and must not be used for other purposes than intended.

The included chemicals are only useable once and are not suitable for consumption.

7. Safety information

When and while working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.

Avoid skin contact.

Adhere to the legal requirements for working with biological material.

For more information, please consult the appropriate material safety data sheets (MSDS). These are available by request on quality@certest.es for each CerTest Product and its components.

If buffer bottles are damaged or leaking, **WEAR GLOVES, AND PROTECTIVE GOGGLES** when discarding the bottles in order to avoid any injuries.

CerTest has not tested the waste generated by the **VIASURE RNA-DNA Extraction Kit** procedures for residual infectious materials. Contamination of the waste with residual infectious materials is unlikely but cannot be excluded completely. Therefore, the waste has to be considered infectious and should be handled and discarded according to local safety regulations.

European Community risk and safety phrases for the critical components of the **VIASURE RNA-DNA Extraction Kit** are listed below as follows:

Lysis Buffer , Wash Buffer I

warning

contains guanidine-hydrochloride;

H302-315-319, P280-305+351+338

Proteinase K

danger

H315-319-334-335 P280-305-351-338-310-405

H302:	Harmful if swallowed.
H315:	Causes skin irritation.
H319:	Causes serious eye irritation.
H334:	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335:	May cause respiratory irritation.
P280:	Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338:	If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses and continue rinsing.
P310:	Immediately call a POISON CENTER or doctor/physician.
P405:	Store locked up.

Emergency medical information can be obtained 24 hours a day from telephone number: 112 (EU)

8. Product characteristics

The **VIASURE RNA-DNA Extraction Kit** provides a fast and efficient way for reliable simultaneous isolation of high quality viral DNA/RNA, bacterial and genomic DNA from a diverse range of starting material. The procedures are suitable for use with human and animal tissues, cells, blood, plasma or serum; either can contain citrate or EDTA (no heparin), urine, and swabs. Best results are obtained with fresh material or immediately frozen. Repeated freezing and thawing of stored samples should be avoided.

Starting Material	Yield	Time for preparation
100 µl fresh or frozen blood, 200 µl fresh or frozen plasma/ serum, urine, cerebrospinal fluid (CSF), suspension of feces. 200 µl supernatant with release swab material, pretreated sputum, BAL, breast milk Additional materials only for veterinarian applications: 20 - 100 µl animal blood 200 µl amniotic fluid	Depending on sample (storage and source)	About 60 min per extraction

The amount of purified DNA and/or RNA in the **VIASURE RNA-DNA Extraction Kit** procedures depend on the sample type, amount and collection date, sample source, transport, storage and the virus titer of starting material. The procedure is designed to avoid sample-to-sample cross-contaminations and allow safe handling of potentially infectious samples.

The DNA/RNA isolation process is based on the interaction of nucleic acids with silica membranes in optimal buffer conditions. After a sample specific lysis using **Lysis Buffer** and **Proteinase K**, optimal binding conditions are adjusted by the addition of Isopropanol. The genomic DNA/RNA binds to the Mini Spin Column. Subsequent to three washing steps of the membrane bound nucleic acids, the nucleic acids are finally eluted in **Elution Buffer**.

Yield and quality of the isolated nucleic acids are suitable for a wide range of molecular-diagnostic detection system. The diagnostic tests should be performed according to manufacturer specifications. Due to the high purity, the isolated DNA/ RNA is ready to use for a broad panel of downstream applications or can be stored at -80°C for subsequent use.

- (RT)-PCR*
- real-time PCR, q(RT)-PCR
- cDNA synthesis
- microarray application

Note: *Systems isolating simultaneously DNA and RNA using buffers adapted for the binding of DNA and RNA, but the optimal binding conditions of RNA and DNA are different, so that such solutions can show a little reduction in sensitivity in comparison to kits optimized to one kind of nucleic acid isolation.*

For further information please contact: +34 976 52 03 54.

9. Principle and Procedure

The **VIASURE RNA-DNA Extraction Kit** procedure comprises following steps:

- Lysis of samples
- DNA/RNA binding to the filter membrane
- Washing the filter bound DNA/RNA and elimination of alcohol
- Elution of DNA/RNA

a. Lysis of samples

Samples are lysed at elevated temperatures in the presence of **Lysis Buffer** (denaturing conditions) and **Proteinase K**.

For bacteria, we recommend a pretreatment with Lysozyme at 37°C before lysis.

b. Binding of nucleic acids to the silica membrane

After adding **Isopropanol** to adjust optimal binding conditions, the lysate will be applied onto the **Mini Spin Column** and the nucleic acids will be adsorbed onto the surface of the Filter membrane as the lysate is drawn through by centrifugation.

c. Removing residual contaminants

Contaminants are efficiently washed away using **Wash Buffer I** and **Wash Buffer II**, while the nucleic acids remain bound to the membrane of the **Mini Spin Column**.

d. DNA/RNA Elution

High quality viral DNA/RNA and genomic/bacterial DNA is eluted from the membrane using **Elution Buffer**. Eluting twice, each time with 100 µl, leads to a little increase of DNA/RNA yield. Usage of small elution volumes may raise the DNA/RNA concentration. Elution volumes should be at least 40 µl of **Elution Buffer**. The volume of eluate recovered may be up to 5 µl less than the volume of elution buffer applied to the **Mini Spin Column**. The volume of eluate recovered depends on the nature of the sample and the amount of Elution Buffer used.

The eluted DNA/RNA is ready to use in different subsequent applications.

10. Important points before starting a protocol

- Immediately upon receipt of the Product, inspect the Product and its components as well as the package for any apparent damages, correct quantities and quality. If there are any unconformities you have to notify CerTest in writing with immediate effect upon inspection thereof.
- If buffer bottles are damaged, contact the CerTest Technical Services or your local distributor. In case of liquid spillage, refer to “Safety Information”. Do not use damaged kit components, since their use may lead to poor kit performance.
- Always change pipette tips between liquid transfers. To avoid cross-contamination, we recommend the use of aerosol-barrier pipette tips.
- When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.
- Discard gloves if they become contaminated.
- Do not combine components of different kits.
- Avoid microbial contamination of the kit reagents.

- To minimize the risk of infections from potentially infectious material, we recommend working under laminar air-flow.
- This kit should be only used by trained personnel.

11. Equipment and reagents to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information please consult the appropriate material safety data sheets (MSDS). (These are available by request on quality@certest.es).

1. Microcentrifuge ≥ 11.000 x *g*
2. Thermomixer (37°C - 95°C)
3. Isopropanol (99.7%), molecular biologic grade
4. Ethanol (96-100%), molecular biologic grade
5. 2.0 ml reaction tubes (optional)
6. Measuring cylinder (250 ml)
7. Vortexer
8. Disposable gloves
9. Pipets and pipet tips with filter
10. 1.5 ml microtubes
11. Centrifuge for 15 or 50 ml (optional)
12. Lysozyme (10 mg/ml) (optional)
13. PBS, pH 7.4 (optional)

The **VIASURE RNA-DNA Extraction Kit** is validated with 2-Propanol; Rotipuran >99.7%, p.a., ACS, ISO (Order no. 6752) from **Carl Roth.**

12. Important indications

12.1 Carrier RNA

The addition of **Carrier RNA** reduces the chance of viral nucleic acid degradation. It minimizes the binding of viral acid to the reaction tubes.

12.2 Handling of Mini Spin Column

Due to the sensitivity of viral DNA/RNA amplification technologies the following precautions are necessary when handling the **Mini Spin Column** to avoid cross-contamination between sample preparation.

1. Carefully apply the sample or solution to the **Mini Spin Column**, pipet the sample onto the filter without wetting the rim of the column.
2. Always change pipet tips between liquid transfers, we recommend the use of aerosol barrier pipet tips.
3. Avoid touching the **Mini Spin Column** membrane with the pipet tip.
4. Close the **Mini Spin Column** before placing it in the microcentrifuge.

13. Yield and quality of purified nucleic acids

Yields of genomic DNA will vary from sample to sample depending on the amount and type of material processed. In addition, the quality of starting material will affect DNA yield.

The amount of purified DNA/RNA from whole blood depends on the leucocytes content, the sample source, transport, storage, and collection date.

The typical yield usually expected from the **VIASURE RNA-DNA Extraction Kit** is about 1 µg DNA.

Please keep in mind, that a small amount of **Carrier RNA** in the eluate will elevate the real genomic DNA content.

Different amplification systems vary in efficiency depending on the total amount of nucleic acid present in the reaction. Eluates derived by this kit will contain **Carrier RNA**, which will greatly exceed the amount of the isolated NA.

Yields of viral nucleic acids isolated from biological samples are usually low concentrated and therefore almost impossible to determine photometrically.*

The kit is suitable for downstream analysis with NAT techniques, for examples qPCR, RT qPCR, LAMP, LCR. Diagnostic assays should be performed according to the manufacturer's instructions.

Quantitative RT-PCR is recommended for determination of viral RNA yield.

* Keep in mind that the **Carrier RNA** (5 µg per 200 µl sample) will account for most of the present NA.

* In Gel Electrophoresis and in Capillary Electrophoresis, DNA extracted with the provided kit looks like degraded cause the kit contains **Carrier RNA**, this is poly A-RNA in fragments of 100 up to 1000 bases. The kit is not dedicated for applications using this kind of analysis.

14. Sampling, storage and preparing of starting materials

14.1 Sampling and storage

For reproducible and high yields appropriate sample storage is essential. Yields may vary from sample to sample depending on factors such as health and age of the donor, kind of sample, collection date, transport and storage conditions. In general, best results are obtained using freshly extracted samples or material that has been immediately frozen and stored at -20°C or -80°C. Avoid multiple thawing and freezing cycles of the sample(s) before isolating the DNA/ RNA.

Blood: Blood samples (stabilized with EDTA or citrate but not heparin) can be stored at room temperature (18-25°C) for 2-3 hours. For short time storage (up to 24 h) samples should be stored at 4-8°C. For long term storage, we recommend to freeze the samples at -20°C or -80°C.

Serum and plasma: After collection and centrifugation, serum and plasma, from blood (treated with anticoagulants like EDTA or citrate, but not with heparin), synovial fluid samples or other cell free body fluids, swabs as well as stool samples can be stored on ice for 1 - 2 hours, for short time (up to 24 h) samples may be stored at -20°C. For long term storage, we recommend freezing samples in aliquots at -80°C. Frozen plasma or serum samples must not be thawed more than once. Multiple thawing and freezing before isolating the viral DNA/ RNA should be avoided. It leads to denaturation and precipitation of proteins, resulting in reduced viral titers and therefore reduced yields of viral nucleic acids. In addition, cryoprecipitate formed during freeze-thawing could make problems. If cryoprecipitate is visible, they should be pelleted by centrifugation at app. 6.800 x *g* for 3 minutes. The cleared supernatant should be aspirated, without disturbing the pellet and processed immediately.

Swab samples, saliva: The protocol works with fresh saliva, prepared swabs as well as with dried

swabs. it is essential, that samples are immediately flash frozen subsequent to the harvesting by using liquid nitrogen and are stored at -80ºC. Viral RNA contained in such deep-frozen samples are stable for months. Viral RNA purification should be processed as soon as possible. Samples can also be stored in the dissolved **Lysis Buffer** for 1 h at room temperature, overnight at 4°C, and for long term storage at -80ºC.

Biopsy material/ tissue: Repeated freezing and thawing of stored samples should be avoided, since this leads to reduced DNA size. Use of poor quality starting material also leads to reduced length and influences yield of purified Nucleic Acid.

Cultivated bacterial: Bacteria have to be pelleted after cultivation. The kit was validated with *Bacillus subtilis* spiked cell-free medium. To perform a quantitative extraction of bacterial DNA, addition of Lysozyme is needed. Please add 5 µl of Lysozyme-solution per 200 µl sample volume to the primary tube before starting the assay.

Urine: The bacteria must be pelleted while the supernatant is completely removed (urea contaminations can inhibit PCR reactions). Best results are obtained with fresh pelleted material or bacteria pellets that has been immediately frozen and stored at -20ºC or -80ºC. The amount of purified DNA from max. 15 - 50 ml urine depends on the included bacteria titre.

Stool samples supernatants: Stool samples typically contain many DNases and RNases which realize quickly DNA and RNA digestion and degradation. The sample may be stored at -80ºC.

Cell culture supernatants: Best results are obtained with fresh material or material that has been immediately frozen and stored at -20ºC or -80ºC after winning of the cell culture supernatant. Repeated freezing and thawing of stored samples can influence the sensitivity.

Amniotic fluid: The protocol works with fresh amniotic fluid and amniotic fluid stored at 4ºC. Best results are obtained using freshly collected samples. The sample can be stored at 4ºC for at least 3 months. For long term storage the samples can be stored at -20ºC.

CerTest will be released of its responsibilities if other sample materials than described in the **Intended Use** are processed or if the sample preparation protocols are changed or modified.

14.2 Preparation of starting materials

1. Extraction of Nucleic acids (NA) from blood, serum, plasma, cell free body fluids, urine, and samples in transport media

This type of sample can be processed directly without any pre-preparations.

Please keep in mind that the first step in the equipment is premixing of samples. Samples have to be at least “pipetable”, mean the presence of clumps and other solid materials leads to clots and prevents a normal workflow of the process.

For blood take care that it is well stored and stabilized with EDTA or Citrate. For blood, please use Protocol 1, for all other materials use protocol 2.

2. Extraction of NA from rinsed liquid from swab samples

a) the sample will also be used for cultivation

Cut off the relevant part of the swab and transfer it into an RNase/DNAse-free 2 ml tube. Add 400 µl physiological saline solutions to the swab and vortex intensely for 2-3 min and incubate for 10 min at RT. Take an aliquot for cultivation. Transfer 200 µl of the rinsed liquid into a **2.0 ml Collection Tube** and follow with the step 1 for NA extraction from bacteria or follow with the steps 1a, b or c for NA extractions from virus in protocol 2.

optional: *If bacterial DNA is processed 20 µl Lysozyme can be added to 200 µl sample, follow the instructions of protocol 2.*

Note: *This does not include any warranty for efficiency of the used cultivation method.*

b) the sample will not be used for cultivation

Cut off the relevant part of the swab and transfer this part into an RNase- and DNAse-free 2 ml tube. Add 400 µl RNase-free water to the swab and vortex intensely for 3 min. Optional, incubate for 3 min at 95ºC. Transfer 200 µl of the rinsed liquid into a **2.0 ml Collection Tube** and follow step 1 for NA extraction from bacteria or follow step 1b or c for NA extractions from virus in protocol 2.

optional: *If bacterial DNA is processed 20 µl Lysozyme can be added to 200 µl sample, follow the instructions of protocol 2.*

3. Extraction of NA from sputum

Transfer 200 µl from the sputum sample into an RNase/DNAse-free tube and add 400 µl saturated Acetylcysteine (ACC) solution to the sample (ratio sample to buffer must be 1:2). Incubate the mixture for 10 min at 95ºC under shaking on the thermomixer to reduce the viscosity. Transfer 200 µl from the mixture into a **2.0 ml Collection Tube** and follow step 1 for NA extraction from bacteria or follow step 1a, b or c for NA extractions from virus in protocol 2.

4. Extraction of NA from slimy tracheal secretes or BAL

a) Non-viscous samples

For isolation of bacterial DNA transfer 1 ml of tracheal secret or BAL into a RNase/DNAse-free tube and centrifuge at 11,000 x *g* for 3 min. Discard / decant the supernatant without disturbing the bacterial pellet: Resuspend the bacterial pellet in 200 µl distilled water or RNAse free water. Transfer the sample into a **2.0 ml Collection Tube** and follow step 1 for NA extraction from bacteria or follow step 2 in protocol 2. For viral NA use the origin sample and follow step 2 in protocol 2

optional: *If bacterial DNA is processed 20 µl Lysozyme can be added to 200 µl sample, follow the instructions of protocol 2.*

b) Viscous sample:

Transfer 1 ml of tracheal secretes or BAL into a RNase- and DNAse-free tube and add 2 ml saturated Acetylcysteine (ACC) solution to the sample (ratio sample to buffer must be 1:2). Incubate the mixture for 10 min at 95ºC to reduce the viscosity and centrifuge at 11,100 x *g* for 3 min.

For isolation of viral NA take a 200 µl aliquote into a **2.0 ml Collection Tube** and follow step 1c in protocol 2.

For isolation of bacterial DNA discard the supernatant without disturbing the bacterial pellet directly. Resuspend the bacterial pellet in 200 µl distilled water or RNase free water and transfer it into a **2.0 ml Collection Tube** and follow step 1a in protocol 2.

optional: *If bacterial DNA is processed 20 µl Lysozyme can be added to 200 µl sample, follow the instructions of protocol 2.*

5. Extraction of NA from supernatant of stool suspension

Transfer 100 µl/ 100 mg stool sample into a 2 ml tube and add 900 µl RNase-free Water. Vortex the sample for 30 s followed by a 1 min centrifugation step at 12,000 x *g*.

Transfer 200 µl of thesupernatant into a **2.0 ml Collection Tube** (prevent the aspiration of swimming particles). For bacterial DNA follow with the step 1c in protocol 2. For viral DNA/RNA follow with the step 1a in protocol 2.

6. Extraction of NA from Biopsy material/ tissue

For genomic DNA extraction, transfer 1 - 10 mg from the tissue biopsy sample into tube and add 200 µl distilled water, 200 µl **Lysis Buffer**, 20 µl **Carrier RNA** (optional) and 20 µl **Proteinase K**.

7. Extraction from bacterial culture

Transfer 1ml of the bacterial culture into a 2.0 ml Collection Tube (not provided). Centrifuge the overnight culture for 2 min at 8000 x *g* and remove completely the supernatant. Resuspend the bacteria pellet in 200 µl PBS Buffer (not provided) and follow step 1a in protocol 2.

8. Extraction of viral DNA/RNA from amniotic fluid

Open the infected egg after cultivation for 5-7 days at 37ºC (depending on the virus). Transfer 200 µl of the allantois liquid into a **2.0 ml Collection Tube** and follow step 1a, b or c in protocol 2.

15. Lysis procedures

For easier handling we recommend to prepare a Master Mix only for the needed amount of samples consisting of **Lysis Buffer**, **Proteinase K** and if required **Carrier RNA**. When preparing the **Master Mix** it is recommended to use a volume of 5 % greater than that required. The Master Mix is stable for at least 2h at RT.

Number of samples	Amount of **Lysis Buffer**	Amount of **Carrier RNA**	Amount of **Proteinase K**	Master Mix total volume
	200 µl / sample	20 µl / sample	20 µl / sample	240 µl / sample
6	1.3 ml	130 µl	130 µl	1.56 ml
8	1.7 ml	170 µl	170 µl	2.04 ml
10	2.1 ml	210 µl	210 µl	2.52 ml
12	2.6 ml	260 µl	260 µl	3 ml
16	3.4 ml	340 µl	340 µl	3.48 ml
20	4.2 ml	420 µl	420 µl	3.96 ml
24	5.0 ml	500 µl	500 µl	4.44 ml
32	6.7 ml	670 µl	670 µl	4.92 ml
40	8.4 ml	840 µl	840 µl	5.4 ml
48	10.0 ml	1000 µl	1000 µl	5.88 ml

16. Extraction control

Extraction control DNA or RNA must be combined with the provided **Carrier RNA** in one mixture.

Add the respective amount of Extraction Control Nucleic Acid to the **Carrier RNA**, if it is in a bigger volume (> 25%) you may replace the according amount of **RNAse free water**.

Notes:

If you only have indication of amount per reaction, please calculate by using eluate and template volume.

If the extraction control is stable in plasma, serum, CSF, urine, respiratory samples, whole blood, stool, transport media, or on dried swabs (e.g., armored RNA), it can alternatively be added to the sample shortly before beginning sample preparation.

If the extraction control is naked DNA or RNA, it is unstable in these specimens must not be added directly to the samples. In carrier RNA, an extraction control stabilized.

Refer to the manufacturer's instructions to determine the optimal amount of extraction control for specific downstream applications. Using an amount other than that recommended may lead to wrong quantification results.

17.Instructions

The following notes are valid for all protocols:

Note: *The DNA/ RNA can also be eluted with a lower (but not lower than 40 µl) or a higher volume of* ***Elution Buffer*** *(depends on the expected yield or needed concentration of the DNA/ RNA). The eluate can contain viral DNA, viral RNA, bacterial or genomic DNA.*

Important: *After extraction place the* ***1.5 ml Collection Tube*** *on ice. For long time storage place the nucleic acids at -20°C or -80°C.*

Note: *The centrifugation steps were made with the* ***Centrifuge 5415 D from Eppendorf.*** *The indicated* ***rpm amounts*** *are referring to this centrifuge.*

Scheme for the Isolation of genomic DNA from blood

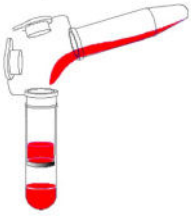
Transfer 100 µl of blood-sample into a **2.0 ml Collection Tube** and add 100 µl **Elution Buffer (Final volume of 200 µl)**.

Add **200 µl Lysis Buffer** and **20 µl Proteinase K,** vortex vigorously for 10 seconds.

Incubate for **15 min at 56°C** while continuously shaking.

Add **260 µl Binding Buffer** and mix by pipetting up and down four times or vortexing.

Incubate the sample **at room temperature** for **5 minutes.**

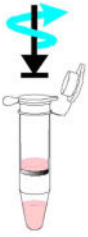


For each sample, take a **Mini Spin Column Set.**
Transfer lysate onto the **Mini Spin Column** without touching the column.
Centrifuge for **1 min** at **11,100 *x g* (11,000 rpm).**
Discard the filtrate and the **RTA Collection Tube**.

Transfer the **Mini Spin Column** in a new **RTA Collection Tube**.
Add **600 µl Wash Buffer I** onto the column.
Centrifuge for **1 min at 11,100 *x g* (11,000 rpm).**
Discard the filtrate and the **RTA Collection Tube**.

Place the **Mini Spin Column** into a new **RTA Collection Tube**.
Add **700 µl Wash Buffer II** onto the column.
Centrifuge for **1 min at 11,100 *x g* (11,000 rpm).**
Discard the filtrate and put the **Mini Spin Column** back into the used **RTA Collection Tube.**

Repeat this washing step once.



Centrifuge for **5 min at 11,100 *x g* (11,000 rpm).** Discard the **RTA Collection Tube.**

Place the **Mini Spin Column** into a **1.5 ml Collection Tube**.
Add 100 - 200 µl of **Elution Buffer** (preheated to 56°C).

Incubate for **1 min** at **room temperature.**

genomic DNA

Centrifuge for **1 min** at **11,100 *x g* (11,000 rpm).**
Discard the **Mini Spin Column.**
Close the **1.5 ml Collection Tube** and store the DNA sample at 4°C, for long term storage at -20°C to -80°C.

Protocol 1: Isolation of genomic DNA from blood

Please read the protocols carefully prior to the start of the preparation procedure!

Important Note: *Prewarm the needed amount of **Elution Buffer to 56°C** for the final elution step. The protocol has been optimized for the isolation of total nucleic acids from 100 µl blood or other body fluids of 200 µl. For samples which have a smaller volume than 200 µl please fill up to a total volume of 200 µl with **Elution Buffer**. Before starting, equilibrate the blood samples at room temperature and verify that they are well mixed.*

1. Sample Lysis

In a **2 ml Collection Tube** mix 100 µl of the blood sample with 100 µl **Elution Buffer**, add 200 µl **Lysis Buffer** and 20 µl **Proteinase K**. Vortex the sample vigorously for 10 seconds or until homogeneous suspension. Place the tubes into a thermomixer and incubate under continuously shaking for 15 minutes at 56°C.

Attention: *By using animal blood the amount of starting material can vary between 20 and 100 µl. Before starting using the kit-please make a dilution series and detect the optimal amount.*

2. Binding of the DNA and RNA

Add 260 µl **Binding Buffer** to the lysate and mix the sample completely by pipetting up and down or by vortexing. Incubate the sample at room temperature for 5 minutes. Transfer the sample into the **Mini Spin Column** put in a **RTA Collection Tube**. Close the cap and centrifuge for 1 minute at 11,100 *x g* (11,000 rpm). Discard the **RTA Collection Tube** with filtrate and place the **Mini Spin Column** into a new **RTA Collection Tube.**

3. First Washing of the Mini Spin Column

Add 600 µl **Wash Buffer I** to the **Mini Spin Column** and centrifuge at 11,100 *x g* (11,000 rpm) for 1 min. Discard the **RTA Collection Tube** with filtrate and place the **Mini Spin Column** into a new **RTA Collection Tube**.

4. Second Washing of the Mini Spin Column

Add 700 µl **Wash Buffer II** to the **Mini Spin Column** and centrifuge at 11,100 *x g* (11,000 rpm) for 1 min. Discard the filtrate and put the **Mini Spin Column** back into the used **RTA Collection Tube.**

5. Repeat this washing step once

6. Ethanol removal

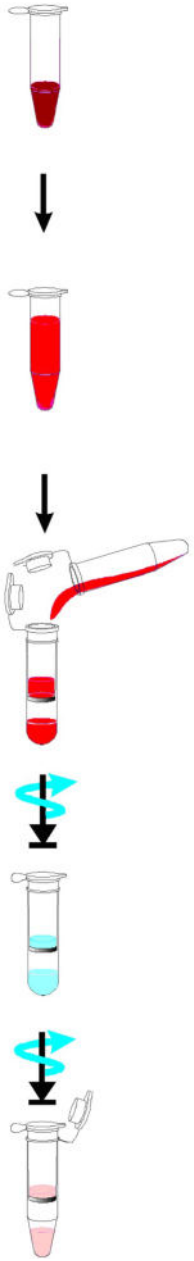
Remove the residual ethanol by final centrifugation for 5 min at 11,100 *x g* (11,000 rpm). Discard the **RTA Collection Tube** with filtrate.

7. Elution of the DNA/ RNA

Place the **Mini Spin Column** into a **1.5 ml Collection Tube** and add 100 - 200 µl of the **Elution Buffer** (prewarmed to 56°C) directly onto the **Mini Spin Column** surface.

Incubate for 1 min at room temperature and centrifuge at 11,100 *x g* (11,.000 rpm) for 1 minute. Discard the **Mini Spin Column**. Close the **1.5 ml Collection Tube** and store the DNA sample at 4 °C, for long term storage at -20°C to -80°C.

Scheme for the simultaneous isolation of pathogen DNA and RNA from all liquid samples



Pathogen NA

Please read protocols prior the start of the preparation carefully

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Transfer 200 µl of origin or pretreated sample into a **2.0 ml Collection Tube.**

For samples which have a smaller volume than 200 µl please adjust to a total volume of 200 µl with **Elution Buffer**.

Only for isolation of bacterial DNA:

Add 20 µl Lysozyme and **20 µl Carrier RNA** and mix vigorously by vortexing.
Incubate for **10 min** at **37°C.**
Add **200 µl Lysis Buffer** and **20 µl Proteinase K,** vortex vigorously.

Only for isolation of viral DNA and RNA:

Add **200 µl Lysis Buffer, 20 µl Carrier RNA** and **20 µl Proteinase K,** vortex vigorously.

Note: *If you handle more than 5 preps at the same time we suggest preparing a Master Mix (as described at point Lysis Procedure). Add 240 µl Master Mix to each sample instead of Carrier RNA, Lysis Buffer and Proteinase K.*

For all:

Incubate for **10 min** at **65°C** and then for **10 min** at **95°C** while continuously shaking.

Add **260 µl Binding Buffer** and mix by vortexing.
Incubate the sample at **room temperature** for **5 minutes.**

For each sample, take a **Mini Spin Column System.**
Transfer lysate onto the **Mini Spin Column** without touching the membrane.
Centrifuge for **1 min at 11,100 x *g* (11,000 rpm).**
Discard the filtrate and the **RTA Collection Tube**.

Transfer the Mini Spin Column in a new RTA Collection Tube.
Add **600 µl Wash Buffer I.**
Centrifuge for **1 min** at **11,100 x g (11,000 rpm).**
Discard the filtrate and the **RTA Collection Tube**.

Transfer the **Mini Spin Column** in a new **RTA Collection Tube.**
Add **700 µl Wash Buffer II** onto the **Mini Spin Column.**
Centrifuge for **1 min** at **11,100 x *g* (11,000 rpm).**
Discard the filtrate and put the **Mini Spin Column** back into the used **RTA Collection Tube.**

Repeat this washing step once.

*Centrifuge for **5 min at 11,100 x g** **(11,000 rpm)**. Discard the **RTA Collection Tube** with filtrate.

Place the **Mini Spin Column** into a **1.5 ml Collection Tube.**
Add 100 - 200 µl of **Elution Buffer** (preheated to 65°C).
Incubate for 1 min at room temperature.
Centrifuge for **1 min at 11,100 x *g* (11,000 rpm).**
Discard the **Mini Spin Column.**
Close the **1.5 ml Collection Tube** and store the DNA/RNA sample at 4ºC. For long term, store the samples at -20 °C to -80°C.

Protocol 2: Simultaneous isolation of pathogen DNA and RNA from all liquid samples

Please read the protocols carefully prior to the start of the preparation procedure!

Important Note: *Prewarm the needed amount of **Elution Buffer to 65°C** for the final elution step. The protocol has been optimized for the isolation of total nucleic acids from body fluids of 200 µl (blood 100 µl). For samples which have a smaller volume than 200 µl please fill up to a total volume of 200 µl with **Elution Buffer**.*

1a) Sample Lysis for bacterial DNA

Note: ***For parallel isolation of bacterial DNA and viral DNA/RNA please use Lysis Protocol 1b)***

Note: ***For bacterial culture**: centrifuge max. 0.5 ml of an overnight culture for 2 min at 5,223 x* g (*8,000 rpm) and remove completely the supernatant. Resuspend the bacteria pellet in 200 µl PBS Buffer (not provided).*

In a **2 ml Collection Tube** mix 200 µl of the sample with 20 µl Lysozyme and 20 µl **Carrier RNA**. Mix vigorously by vortexing. Incubate for 10 min at 37°C. Add 200 µl **Lysis Buffer** and 20 µl **Proteinase K**. *(See note at the end of the protocol).

Vortex the sample vigorously for 10 seconds. Place the microtube into a thermomixer and incubate under continuously shaking for 10 to 15 minutes at 65°C and then optional for 10 min at 95°C.

1b) Sample Lysis for simultaneous isolation of bacterial DNA and viral NA

In a **2 ml Collection Tube** mix 200 µl of the sample with 200 µl **Lysis Buffer**, 20 µl **Carrier RNA** and 20 µl **Proteinase K**. *(See note at the end of the protocol).

Vortex the sample vigorously for 10 seconds. Place the tube into a thermomixer and incubate under continuous shaking for 10 minutes at 65°C and then for 10 minutes at 95°C.

Before you add Lysozyme to the mixture, take care that the sample is cooled down to < 40°C.
Add 20 µl **Lysozyme** to the lysed sample and incubate for 10 min under shaking at RT.

1c) Sample Lysis for virus NA:

In a **2 ml Collection Tube** mix 200 µl of the sample with 200 µl **Lysis Buffer**, 20 µl **Carrier RNA** and 20 µl **Proteinase K**. *(See note at the end of the protocol).

Vortex the sample vigorously for 10 seconds. Place the tube into a thermomixer and incubate under continuous shaking for 10 minutes at 65°C and then for 10 minutes at 95°C.

***Note stool samples**: These samples have to be diluted 1:10 with **RNAse free water**. Vortex the sample for 30 sec. Centrifuge the sample for 1 min at 13,362 x g (12,000 rpm) and transfer the supernatant in a new tube (not provided).*

2. Binding of the DNA and RNA

Add 260 µl **Binding Buffer** to the lysed sample and mix the sample completely by pipetting up and down or by vortexing. Incubate the sample at room temperature for 5 minutes. Transfer the sample into the **Mini Spin Column** put into a **RTA Collection Tube**. Close the cap and centrifuge for 1 minute at 11,100 *x g* (11,000 rpm). Discard the **RTA Collection Tube** with filtrate and place the **Mini Spin Column** into a new **RTA Collection Tube**.

3. First washing of the Mini Spin Column

Add 600 µl **Wash Buffer I** to the **Mini Spin Column** and centrifuge at 11,100 x *g* (11,000 rpm) for 1 min. Discard the **RTA Collection Tube** with filtrate and place the **Mini Spin Column** into a new **RTA Collection Tube**.

4. **Second washing of the Mini Spin Column**

Add 700 µl **Wash Buffer II** to the **Mini Spin Column** and centrifuge at 11,100 x *g* (11,000 rpm) for 1 min. Discard the filtrate and put the **Mini Spin Column** back into the used **RTA Collection Tube**.

5. **Repeat this washing step once.**

6. **Ethanol removal**

Remove the residual ethanol by final centrifugation for 5 min at 11,100 x *g* (11,000 rpm). Discard the **RTA Collection Tube** with filtrate.

7. **Elution of the DNA/ RNA**

Place the **Mini Spin Column** into a **1.5 ml Collection Tube** and add 100 - 200 µl of the **Elution Buffer** (prewarmed to 65°C) directly onto the **Mini Spin Column** surface.

Incubate for 1 min at RT and centrifuge at 11,100 *x g* (11,000 rpm) for 1 minute. Discard the **Mini Spin Column**. Close the **1.5 ml Collection Tube** and store the sample at -20 °C to -80°C.

***Note:** If you handle more than 5 preps at the same time we suggest preparing a Master Mix (as described at point Lysis Procedure).*
Add 240 µl Master Mix to each sample instead of Carrier RNA, Lysis Buffer and Proteinase K.

Additional protocol: Simultaneous isolation of nucleic acids* from tissue biopsies

Please read the protocols carefully prior to the start of the preparation procedure

Important Note: *Prewarm the needed amount of* ***Elution Buffer*** *to 65°C for the final elution step. Switch on heating blocks (e.g. thermomixer) to 65°C and 95°C.*

1. Sample Lysis

Transfer 1-10 mg from the tissue biopsy sample into a **2.0 ml Collection Tube** and add 200 µl distilled water or PBS, 200 µl **Lysis Buffer**, 20 µl **Carrier RNA** and 20 µl **Proteinase K** to each sample.

Note: If you handle more than 5 preps at the same time we suggest preparing a Master Mix (as described at point Lysis Procedure). Add 240 µl Master Mix to each sample instead of **Carrier RNA**, **Lysis Buffer** and **Proteinase K**.

Note: The addition of **Carrier RNA** to the sample is here optional.

Place the Tube into a thermomixer and incubate under continuous shaking for **10 minutes at 65°C** and then for **10 minutes at 95°C.**

Note: Lysis time may be increased if the lysis is incomplete.

Note: For bacterial DNA, before you add Lysozyme to the mixture, take care that the sample is cooled down to < 40°C. Add 20 µl Lysozyme to the lysed sample and incubate for 10 min under shaking at RT.

Important: A longer lysis time could reduce the final yield and quality of some viral RNA species.

After lysis, centrifuge the sample at max. speed for 1 minute to spin down unlysed material. Transfer the cleared supernatant completely into a 1.5 ml microtube (not provided).

2. Binding of the DNA and RNA

Add 260 µl **Binding Buffer** to the sample and mix completely by pipetting up and down or by vortexing. Transfer the sample into the **Mini Spin Column** put into a **RTA Collection Tube**. Close the cap and centrifuge for 2 minutes at 11,000 x *g* (11,000 rpm). Discard the **RTA Collection Tube** with filtrate and place the Mini Spin Column into a new **RTA Collection Tube.**

3. First Washing of the Mini Spin Column

Add 600 µl **Wash Buffer I** to the Mini Spin Column and centrifuges at 11,000 x *g* (11,000 rpm) for 1 min. Discard the **RTA Collection Tube** with filtrate and place the **Mini Spin Column** into a new **RTA Collection Tube**.

4. Second Washing of the Mini Spin Column

Add 700 µl **Wash Buffer II** to the **Mini Spin Column** and centrifuge at 11,000 *x g* (11,000 rpm) for 1 min. Discard the **RTA Collection Tube** with filtrate and place the **Mini Spin Column** into a new **RTA Collection Tube.**

(*) nucleic acids: include genomic DNA, bacterial DNA, viral DNA and viral RNA

5. Ethanol removal

Remove the residual ethanol by final centrifugation for 5 min at maximum speed. Discard the **RTA Collection Tube** with filtrate.

6. Elution of the DNA/ RNA

Place the **Mini Spin Column** into a **1.5 ml Collection Tube.** Add 60 µl of the **Elution Buffer** (prewarmed to 65°C) directly onto the Mini Spin Column surface. Incubate for 3 minutes at RT and centrifuge at 11,000 x *g* (11,000 rpm) for 1 minute. Discard the **Mini Spin Column**. Close the **1.5 ml Collection Tube** and store the sample at -20 °C to -80°C.

Troubleshooting

Problem	Probable cause	Comments and suggestions
Common errors	**Proteinase K** volume/concentration too low Reagent / Buffer volume too low	Make sure that you have resuspended the lyophilized **Proteinase K** with the appropriate volume of water before use.
	Lysate not completely passed through silica membrane	Centrifuge at full speed for 1 minute or until all the lysate has passed through the membrane of the column.
Low yield or concentration of extracted DNA/RNA	No addition/ too much isopropanol added to **Wash Buffer I**	Ensure that the **Wash Buffer I** has been filled up with isopropanol properly as indicated before in this manual.
	No addition/ too much ethanol added to **Wash Buffer II**	Ensure that the **Wash Buffer II** has been filled up with ethanol properly as indicated before in this manual.
	Incorrect storage of starting material	Ensure that the storage of starting material was correct. Avoid multiple freezing and thawing cycles of the material.
	Insufficient lysis	Ensure that samples have been mixed properly with Lysis Buffer and Proteinase K.
	Deficient NA elution	*Prewarm the **Elution Buffer** as described before in this manual.* Add the **Elution Buffer** in the center of the membrane.
Degraded or sheared DNA/RNA	Sample stored incorrectly	Samples should be stored at 4ºC, -20ºC or -80ºC
	Old material	Ensure that the starting material is fresh or stored under appropriate condition (for long time storage at -20°C o -80ºC). Avoid multiple thawing and freezing cycles of the material. Old material often contains degraded DNA/RNA For the extraction of nucleic acids from old samples or coagulated blood samples it is recommended to incubate samples with Proteinase K for 30 minutes.

	Combination of reagents from different kits	Please make sure that only reagents belonging to one kit type are used. A combination of reagents belonging to different kit types will not work.

Appendix

General notes on handling DNA

Nature of DNA

The length and delicate physical nature of DNA requires careful handling to avoid damage due to shearing and enzymatic degradation. Other conditions that affect the integrity and stability of DNA include acidic and alkaline environments, high temperature, and UV irradiation. Careful isolation and handling of high molecular weight DNA is necessary to ensure compatibility with various downstream applications. Damaged DNA could perform poorly in applications such as genomic Southern blotting, and long-template PCR.

Storage of DNA

A working stock of DNA can be stored at 2 - 4˚C for several weeks. For long term storage DNA should be stored at -20˚C but storing at -20°C can cause shearing, particularly if the DNA is exposed to repeated freeze-thaw cycles.

Note that the solution in which the nucleic acid is eluted in will affect its stability during storage. Pure water lacks buffering capacity and an acidic pH may lead to acid hydrolysis. Tris or Tris-EDTA buffer contains sufficient buffering capacity to prevent acid hydrolysis.

Drying, dissolving and pipetting DNA

Avoid over drying genomic DNA after ethanol precipitation. It is better to let it air dry than to use a vacuum, although vacuum drying can be used with caution.
Avoid vigorous pipetting. Pipetting genomic DNA through small tip openings causes shearing or nicking. One way to decrease shearing of genomic DNA is to use special tips that have wide openings designed for pipetting genomic DNA.

General notes on handling RNA

RNA is far less stable than DNA. It is very sensitive to degradation by endogenous RNases in the biological material and exogenous RNases which are permanently present everywhere in the lab. To achieve satisfactory qualitative and quantitative results in RNA preparations, contaminations with exogenous RNases have to be reduced as much as possible. Avoid handling bacterial cultures, cell cultures, or other biological sources of RNases in the same lab where the RNA purification is to be carried out.

All glassware should be treated before use to ensure that it is RNase free. Glassware should be cleaned with detergent, thoroughly rinsed and oven baked at 240ºC for four or more hours before use. Autoclaving alone will not completely inactivate many RNases. Oven baking will both inactivate RNases and ensure that no other nucleic acids (such as Plasmid DNA) are present on the surface of the glassware. You can also clean glassware with 0.1% DEPC (diethyl pyrocarbonate). The glassware must stand 12 hours at 37ºC and then be autoclaved or heated to 100ºC for 15 minutes to remove residual DEPC.

- **Electrophoresis tanks** should be cleaned with detergent solution (e.g. 0.5% SDS), thoroughly rinsed with RNase free water, and then rinsed with ethanol and allowed to dry.
- **Non-disposable plasticware** should be treated before use to ensure that it is RNase free.
- All **buffers** must be prepared from DEPC-treated RNase free ddH_2O.
- Change gloves frequently and keep tubes closed.
- Reduce the preparation time as much as possible.
- **Use only sterile, disposable polypropylene tubes** throughout the procedure. (These tubes are generally RNase free.)
- Keep isolated RNA on ice.

This kit should only be used by personnel trained in laboratory practice.

Storage of RNA
Purified RNA can be stored -80ºC and is stable for months and years.

Quantification
Quantification of DNA and RNA from this assay must be done by means of qPCR or Reverse Transcriptase qPCR. All other methods will be disturbed by the included **Carrier RNA** as well as DNA or RNA which is co-purified.

VIASURE

VIASURE