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USER MANUAL

RTP® DNA/ RNA Virus Mini Kit

for simultaneous purification of viral DNA and RNA from serum and plasma samples, cell culture supernatants and other cell-free body fluids (e.g. urine), swabs, tissue biopsies and stool suspension

CE IVD

REF 1040100300

Invitek Molecular GmbH · D-13125 Berlin

Instruction for the RTP® DNA/ RNA Virus Mini Kit

The **RTP® DNA/ RNA Virus Mini Kit** is a tool for simultaneous isolation of viral DNA and RNA from human and animal serum and plasma samples, cerebrospinal fluid, cell culture supernatants and other cell-free body fluids, like urine, supernatant of stool suspension as well as from swabs or tissue biopsies for *in-vitro*-diagnostic purposes using a spin-filter format. Fresh or frozen plasma or serum from blood treated with anticoagulants like EDTA or citrate, *but not with heparin*. The customer convenient RTP® technology simplifies the process handling, reduces the handling steps with infectious material and allows a process monitoring.

Due to the high purity, the isolated viral DNA and RNA is ready to use for a broad panel of downstream applications or can be stored at – 80 °C for subsequent use.

The kit is neither suitable for isolation of viral DNA or RNA from whole blood or blood stains, nor for isolation of RNA or DNA from bacteria, fungi, plants.

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

Kit contents of RTP® DNA/ RNA Virus Mini Kit

	250 extractions
Catalogue No.	1040100300
Extraction Tubes	5 x 50
Binding Solution (fill with 99.7% Isopropanol)	empty bottle (final volume 120 ml)
Wash Buffer R1	80 ml (final volume 160 ml)
Wash Buffer R2	50 ml (final volume 250 ml)
Elution Buffer R	30 ml
RTA Spin Filter Set	5 x 50
RTA Receiver Tubes	15 x 50
Elution Tubes	5 x 50
Manual	1
Initial steps	Fill 120 ml 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle Add 80 ml of 96 – 100 % ethanol to the bottle **Wash Buffer R1**, mix thoroughly and always keep the bottle firmly closed Add 200 ml of 96 – 100 % ethanol to the bottle **Wash Buffer R2**, mix thoroughly and always keep the bottle firmly closed!

Symbols

 Manufacturer

 Lot number

Attention: *Do not combine components of different kits, unless the lot numbers are identical!*

 Catalogue number

 Expiry date

 Consult operating instructions

 Temperature limitation

 Do not reuse

 Humidity limitation

Storage

All buffers and kit contents of the **RTP® DNA/ RNA Virus Mini Kit** including the **Extraction Tube** (incl. Lysis Buffer, Proteinase K, Carrier RNA and Internal Control DNA) should be stored at room temperature and are stable for at least 12 months. Store the Kit especially the Extraction Tubes in a dry environment, the Extraction Tubes must be protected from humidity

Room temperature (RT) is defined as range from 15 - 30°C.

Before every use, make sure that all components have room temperature. If there are any precipitates within the provided solutions solve these precipitates by warming carefully (up to 30°C).

Wash Buffers charged with ethanol should be appropriately sealed and stored at room temperature.

Binding Solution (Isopropanol) should be appropriately sealed and stored at room temperature.

Quality control and product warranty

Invitek Molecular warrants the correct function of the **RTP® DNA/ RNA Virus Mini Kit** for applications as described in this manual. Purchaser must determine the suitability of the Product for its particular use. Should any Product fail to perform the applications as described in the manual, Invitek Molecular will check the lot and if Invitek Molecular investigates a problem in the lot, Invitek Molecular will replace the Product free of charge.

Invitek Molecular reserves the right to change, alter, or modify any Product to enhance its performance and design at any time.

In accordance with Invitek Molecular's EN ISO 13485 certified Quality Management System the performance of all components of the **RTP® DNA/ RNA Virus Mini 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 **RTP® DNA/ RNA Virus Mini Kit** or other Invitek Molecular products, please do not hesitate to contact us. A copy of Invitek Molecular's terms and conditions can be obtained upon request or are presented at the Invitek Molecular webpage www.invitek-molecular.com.

For technical support or further information please contact:

from Germany	**+49-(0)30-9489-2901/ 2910**
from abroad	**+49-(0)30-9489-2907**
	or contact your local distributor.

Intended use

The **RTP® DNA/ RNA Virus Mini Kit** is the ideal tool for reliable and fast simultaneous isolation of high quality viral DNA and RNA from fresh or frozen human or mammalian serum, plasma, cerebrospinal fluid, cell culture supernatants and other cell free body fluids as well as from swabs or tissue biopsies.
For reproducible high yields an appropriate sample storage and quick operation under the rules for RNA and DNA operation is essential. The purified viral DNA and/or RNA is ready to use for *in vitro* diagnostic analysis only.

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

THE PRODUCT IS ITDENDED FOR USE BY PROFESSIONALS ONLY, SUCH AS TECHNICIANS, PHYSICIANS AND BIOLOGISTS TRAINED IN MOLECULAR BIOLOGICAL TECHNIQUES. It is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of DNA/ RNA 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 complies with EU Directive 98/79/EC on in vitro medical devices. However, 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.

Product use limitation

The kits are neither suitable for isolation of viral DNA or RNA from whole blood or blood stains, nor for isolation of RNA or DNA from bacteria, fungi, plants.

The included chemicals are only useable once.

Differing of starting material or flow trace may lead to inoperability; therefore, neither a warranty nor guarantee in this case will be given, neither implied nor express.

The user is responsible to validate the performance of the Invitek Molecular Product for any particular use. Invitek Molecular does not provide for validation of performance characteristics of the Product with respect to specific applications.

Invitek Molecular products may be used e.g. in clinical diagnostic laboratory systems under following conditions:

- If used in the US, based on the condition that the complete diagnostic system of the laboratory has been validated pursuant to CLIA' 88 regulations.
- For other countries based on the condition that the laboratory has been validated pursuant to equivalents according to the respective legal basis.

.All Products sold by Invitek Molecular are subject to extensive quality control procedures (according to EN ISO 13485) and are warranted to perform as described herein. Any problems, incidents or defects shall be reported to Invitek Molecular 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 purposes other than intended.

The Product with its contents is unfit for consumption.

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 online in convenient and compact PDF format at www.invitek-molecular.com for each Invitek Molecular 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.

Invitek Molecular has not tested the liquid waste generated by the **RTP® DNA/ RNA Virus Mini Kit** procedure for residual infectious materials. Contamination of the liquid waste with residual infectious materials is highly unlikely, but cannot be excluded completely. Therefore, liquid waste must be considered infectious and be handled and discarded according to local safety regulations.

European Community risk and safety phrases for the components of the **RTP® DNA/ RNA Virus Mini Kit** to which they apply is listed are listed below as follows:

Extraction Tubes

Warning

H302-H315-H319-H335-H411-P280-P305+P351+P338-EUH208

Wash Buffer R1

Warning

H302-H332-H412-P280-P305+P351+P338-EUH032

H302: Harmful if swallowed.
H315: Causes skin irritation.
H319: Causes serious eye irritation.
H332: Harmful if inhaled.
H335: May cause respiratory irritation.
H411: Toxic to aquatic life with long lasting effects.
H412: Harmful to aquatic life with long lasting effects.
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, if present and easy to do. Continue rinsing.
EUH208: Contains Proteinase, Tritirachium album-Serine. May produce an allergic reaction.
EUH032: Contact with acids liberates very toxic gas.

Emergency medical information can be obtained 24 hours a day from infotrac:

outside of USA: 1 – 352 – 323 – 3500
in USA : 1 – 800 – 535 – 5053

Product characteristic of the RTP® DNA/ RNA Virus Mini Kit

Starting material	Yield	Time
up to 200 µl cell free body fluids; swab material, cell culture supernatants up to 400 µl rinse liquid from swab 1 x 10^6 mammalian cells max. 10 mg tissue sample 100 µl/ 100 mg stool sample	depends on the sample (storage and source) ***Note:*** The added Carrier RNA will account for most of the eluted RNA. Quantitative RT-PCR is recommended for determination of the viral RNA or DNA yield.	20 minutes

The **RTP® DNA/ RNA Virus Mini Kit** provides a fast and efficient way for reliable simultaneous isolation of high quality viral DNA and RNA from a diverse range of starting material. The procedures are suitable for use with plasma or serum; either can contain citrate or EDTA (no heparin) and other samples. Samples can be fresh, lyophilized or frozen, provided they have not been frozen and thawed more than once.
The amount of purified viral DNA and RNA in the **RTP® DNA/ RNA Virus Mini Kit** procedure depend on the sample type, the virus titer, sample source, transport, storage, and age.

The kits use the patented RTP® technology, whose special feature is an **Extraction Tube** containing preformulated solid lysis reagent, Proteinase K and Carrier Nucleic Acids**.** The technology allows the reduction of reagent preparation steps and of handling steps with infectious material.

Using the **RTP® DNA/ RNA Virus Mini Kit,** all types of samples are transferred into the Extraction Tubes together with ddH_2O to adjust a final sample volume of 400 µl. The prefilled buffer and enzymes lyse the samples, stabilize the viral nucleic acids and enhance the selective viral RNA and DNA adsorption to the membrane in the RTA Spin Filter. Contaminants are removed by repeated washing steps and the purified viral DNA and/ or RNA can be eluted in a small volume of **Elution Buffer R**.

The advantage of the kit results from the simultaneous isolation of nucleic acids from DNA and RNA viruses. That enables the user to test the sample for all kinds of RNA and DNA viruses, which are of interest after one preparation.

Yield and quality of isolated viral DNA and RNA is suitable for any molecular-diagnostic detection system. The diagnostic tests should be performed according to manufacturer's specifications. Due to the high purity, the isolated viral 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 (quantitative (RT)-PCR, like TaqMan und LightCycler technology)
- cDNA syntheses
- Mircoarray-application

The purification procedure is rapid and does not require a phenol/ chloroform extraction or ß-Mercaptoethanol. Only a minimum of manual work by the user is necessary. The procedure is designed to avoid sample-to-sample cross-contaminations and allow safe handling of potentially infectious samples. The procedure is highly suited for simultaneous processing of multiple samples. Traditional time-killing procedures can be replaced using the **RTP® DNA/ RNA Virus Mini Kit.** All kit components can be stored at room temperature.

Invitek Molecular also offers in combination with magnetic bead the **InviMag® Virus DNA/ RNA Mini Kit/ KFml.**

Note: *Systems isolating simultaneously DNA and RNA from viruses 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 technical support or further information please contact: **+49 - (0)30-9489-2901/ 2907/ 2910 or your local distributor.**

Internal controls/ Extraction control

Internal Controls (IC) from the PCR assay provider can be used as extraction controls if the fragments are longer than 100 bp. In this case, they have to be added after finalization of the lysis step.

Attention: Do not add directly these Internal Controls to the sample!

Principle

The **RTP® DNA/ RNA Virus Mini Kits** procedure comprises following steps:

1. lysis of the virus particles
2. adjustment of the binding conditions
3. binding the viral nucleic acids in the lysate to the membrane of a Spin Filter RTA
4. washing of the membrane and elimination of contaminants and ethanol
5. elution of the viral nucleic acids

This manual contains 4 protocols.

Sampling and storage of starting material

Best results are obtained using freshly extracted samples. As long as the samples are not shock frosted with liquid nitrogen or are incubated with RNase inhibitors or denaturing reagents, the viral RNA is not secured. Therefore, it is essential, that samples are immediately flash frozen subsequent to the harvesting by using liquid nitrogen and are stored at -80°C. Viral RNA contained in such deep frozen samples is stable for months. Viral RNA purification should be processed as soon as possible. Samples can also be stored in the dissolved Lysis Buffer in the Extraction TubeI or II for 1 h at room temperature, overnight at 4°C, and for long-term storage at –80°C. Storage under deep frozen conditions is recommended.

Serum, plasma, urine, cerebrospinal fluid or other cell free body fluids, as well as cell culture supernatants, swabs, and stool samples can be stored on ice for 1 - 2 hours, for short time (up to 24h) samples may be stored at -20°C. For long-term storage, we recommend freezing samples at –80°C. Multiple thawing and freezing before isolating the viral RNA should be avoided.

Serum and plasma (and other cell free body fluids)

Following centrifugation, plasma or serum from blood treated with anticoagulants like EDTA or citrate, *but not with heparin*, can be stored at 2–8°C for up to 6 hours. For long-term storage, freezing at –20°C to –80°C in aliquots is recommended. Repeated freezing and thawing cycles must be avoided because denaturation and precipitation of proteins result in a decrease of the virus titer and thereby reduce the yield of the extracted viral RNA. Occurring cryoprecipitates can be pelleted by briefly centrifuging (6.800 x g for 3 minutes). The cleared supernatant should be removed, without disturbing the pellet, and processed immediately. This step will not reduce viral titers.

Tissue samples (biopsy material or frozen section)

Best results are obtained with fresh material or material that has been immediately flash frozen and stored at –20°C or –80°C. Repeated freezing and thawing of stored samples should be avoided, since this leads to reduced RNA yield. Use of poor quality starting material influences the RNA yield, too. The amount of purified RNA in the **RTP® DNA/ RNA Virus Mini Kit** procedure using up to 20 mg tissue sample, depends on kind of starting material. The thawing process could be proceed, e.g. directly in the **Extraction Tubes.**

Cell culture supernatants

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

Swabs

The protocol works with fresh prepared swabs or rinsed liquid from swabs or mouth brushes. Best results are obtained with fresh material or material that has been immediately frozen and stored at –20°C or –80°C.

Invitek Molecular 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.

Procedure

Lysis
Samples are lysed under denaturing conditions at elevated temperatures. Due to the strong denaturing lyses conditions in the presence of **Proteinase K** and the **Lysis Buffer** cells are quickly broken and RNases and DNases are inactivated simultaneously. The viral RNA and DNA are secured. The addition of **Carrier RNA** (provided in the Extraction Tube) is necessary for the enhancement of viral DNA/ RNA recovery so a very small number of viral DNA/ RNA molecules will also be purified. **Carrier RNA** also stabilizes nucleic acids in samples with very small nucleic acid concentrations.

Binding viral RNA
After adding **Binding Solution** to optimize the binding of viral DNA/ RNA to the RTA Spin Filter membrane, the lysate will be applied onto the RTA Spin Filter and the viral DNA/ RNA are bound to the surface of the RTA Filter membrane as the lysate is drawn through by centrifugation.

Removing residual contaminants
Contaminants are efficiently washed away using **Wash Buffer R1** and **R2**, while the viral DNA/ RNA remain bound to the membrane of the RTA Spin Filter.

Elution
High quality viral DNA/ RNA is eluted from the membrane using **Elution Buffer R** or RNase free water. The eluted DNA/ RNA is ready to use in different subsequent applications.

Important notes

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 Invitek Molecular in writing with immediate effect upon inspection thereof. If buffer bottles are damaged, contact the Invitek Molecular Technical Services or your local distributor. In case of liquid spillage, refer to “Safety Information” (see page 7). Do not use damaged kit components, since their use may lead to poor kit performance.

1. Always change pipet tips between liquid transfers. To avoid cross-contaminations, we recommend the use of aerosol-barrier pipet tips.
2. All centrifugation steps are carried out at room temperature.
3. When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.
4. Discard gloves if they become contaminated.
5. Do not combine components of different kits unless the lot numbers are identical.
6. Avoid microbial contamination of the kit reagents.
7. To minimize the risk of infections from potentially infectious material, we recommend working under laminar air-flow until the samples are lysed.
8. This kit should only be used by trained personnel.

Important indications

Preparing viral RNA
When preparing viral RNA, work quickly during the manual steps of the procedure.
The Lysis Buffer in the Extraction Tube simplifies viral RNA isolation by combining efficient lysis of the starting material and the inactivation of exogenous and endogenous RNases. Special care should be taken to avoid contaminations with RNases when handling Elution Buffer R.

Storing samples
Frozen serum or plasma samples must not be thawed more than once. Repeated freeze –thawing 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 will clog the RTA Spin Filter membrane.

Carrier RNA
Carrier RNA serves two purposes. Firstly, it enhances the binding of viral acids to the RTA Spin Filter membrane, especially if there are very few target molecules in the sample. Secondly, the addition of large amounts of Carrier RNA reduces the chance of viral nucleic acid degradation in the rare event that RNase or DNase molecules are not denaturated by the salts and detergents in the Lysis Buffer in the Extraction Tube.

Internal Control
The use of an internal control is recommended when using the **RTP® Virus DNA/ RNA Mini Kit** in combination with diagnostic amplification systems.

Eluting viral RNA and DNA
For downstream applications, that require small starting volumes, elution volume may be reduced to 40 µl Elution Buffer R.

The volume of eluate recovered may be up to 5 µl less than the volume of elution buffer applied to the RTA Spin Filter. The volume of eluate recovered depends on the nature of the sample.

Handling of RTA Spin Filter
Due to the sensitivity of viral DNA/ RNA amplification technologies the following precautions are necessary when handling RTA Spin Filter to avoid cross-contamination between sample preparations.

1. carefully apply the sample or solution to the RTA Spin Filter, pipet the sample into 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 RTA Spin Filter membrane with the pipet tip

Yield and quality of viral DNA/ RNA

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.*

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

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.

**In Gel Electrophoresis and in Capillary Electrophoresis, RNA 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.*

Preparing buffers

250 viral DNA/ RNA-extractions:
Fill 120 ml 99.7% Isopropanol (molecular biologic grade) into the empty bottle Add 80 ml 96-100% ethanol to each bottle **Wash Buffer R1**. Add 200 ml 96-100% ethanol to each bottle **Wash Buffer R2**.

Harvesting 1x10^6 mammalian cells

Cells grown in suspension:	Spin up to 1 x 10^6 cells for 5 min at 240 x g (1.500 rpm). Discard the supernatant and remove all media completely.
Cells grown in a monolayer:	In large culture vessels (dishes > Ø 35 mm, flasks > 12.5 cm^2) detach cells by trypsination. Transfer the cells to a centrifuge tube and sediment by centrifugation at 240 x g (1.500 rpm) for 5 minutes. Remove the supernatant completely. In small culture vessels (96-, 24-, 12-, 6-well plates, Ø 35 mm dishes, 12.5 cm^2 flasks) discard the media completely and continue with the lysis immediately.

Important: *Some specific cell culture media may inhibit lysis efficiency or downstream reactions.*

For special comments on the diverse **sample preparation procedures** see the respective protocol on page 16.

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's, please consult the appropriate material safety data sheets (MSDS). (See our webpage: www.invitek-molecular.com)

- Microcentrifuge ≥ 11.000 x g (≥ 11.000 rpm)
- Thermomixer (65°C - 95°C)
- ddH_2O
- Ethanol (96-100%)
- 2.0 ml reaction tubes (optional)
- Measuring cylinder (250 ml)
- Disposable gloves
- Pipet with tips
- 1.5 ml reaction tubes
- Isopropanol *

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

*** Possible suppliers for Isopropanol:**

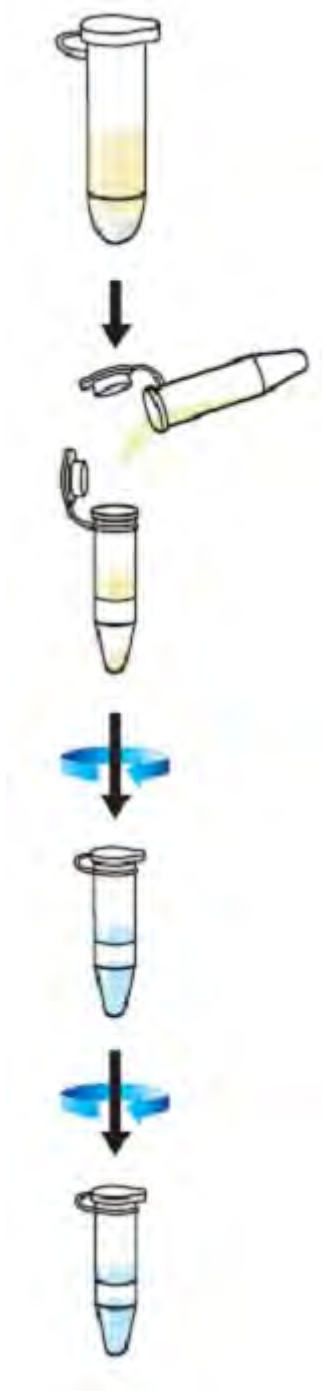
Carl Roth
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Order no. 6752

Applichem
2-Propanol für die Molekularbiologie
Order no. A3928

Fa. Sigma
2-Propanol
Order no. 59304-1L-F

Scheme of the RTP® DNA/ RNA Virus Mini Kit

Please work quickly and perform all extraction steps at room temperature (RT)!



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

transfer 200 µl sample into the provided **Extraction Tubes**
add 200 µl **dd H_2O**
for samples which have a smaller volume than 200 µl please fill up to a total volume of 400 µl with ddH_2O
incubate for 15 minutes at 65°C in a thermomixer
incubate for 10 minutes at 95°C in a thermomixer (optional)

for optimal binding conditions add 400 µl **Binding Solution** and mix the sample completely by pipetting up and down or by vortexing

transfer the sample on the RTA Spin Filter
incubate for 1 min
centrifuge for 2 min at 11.000 x g (11.000 rpm)
discard the flow-through with the RTA Receiver Tube
put the RTA Spin Filter in a new RTA Receiver Tube

pipet 500 µl **Wash Buffer R1** onto the RTA Spin Filter
centrifuge for 1 min at 11.000 x g (11.000 rpm)
discard the flow-through and the RTA Receiver Tube
transfer the RTA Spin Filter into a new RTA Receiver Tube

pipet 700 µl **Wash Buffer R2** onto the RTA Spin Filter
centrifuge for 1 min at 11.000 x g (11.000 rpm)
discard the flow-through and the RTA Receiver Tube
transfer the RTA Spin Filter into a new RTA Receiver Tube

to eliminate any traces of ethanol, centrifuge again for 4 min at maximum speed
discard the RTA Receiver Tube

transfer the RTA Spin Filter into a RNase free 1.5 ml Elution Tube
pipet 60 µl of **Elution Buffer R** (preheated to 65°C) directly onto the membrane of the RTA Spin Filter,
incubate for 3 min
centrifuge for 1 min at 11.000 x g (11.000 rpm)
discard the RTA Spin Filter and place the eluted viral DNA/ RNA immediately on ice

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 R (depends on the expected yield or needed concentration of the DNA/ RNA).*
The eluate contains viral DNA and/ or viral RNA.
If the starting material is e.g. tissue the eluate contains further genomic DNA.

Important *After extraction, place the Elution 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.*

Protocol 1: Simultaneous isolation of total nucleic acids (DNA and RNA) from cell free body fluids

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

Important Note: *Prewarm the needed amount of **Elution Buffer R** 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. For samples which have a smaller volume than 200 µl please fill up to a total volume of 400 µl with ddH_2O.*

1. Sample Lysis
Mix 200 µl of the sample with 200 µl of ddH_2O.
Transfer the sample into the provided Extraction Tube. Close the cap and vortex shortly.
Place the Extraction Tubes into a thermomixer and incubate under continuously shaking for 15 minutes at 65°C and for 10 minutes at 95°C, this leads to higher sensitivity.

2. Binding of the DNA and RNA
Add 400 µl **Binding Solution** to the provided **Extraction Tube** and mix the sample completely by pipetting up and down or by vortexing. Transfer the sample into the RTA Spin Filter Set.
Close the cap and centrifuge for 2 minutes at 11.000 x g (11.000 rpm).
Discard the RTA Receiver Tube with filtrate and place the RTA Spin Filter into a new RTA Receiver Tube.

3. First Washing of the RTA Spin Filter
Add 500 µl **Wash Buffer R1** to the RTA Spin Filter and centrifuge at 11.000 x g (11.000 rpm) for 1 minute.
Discard the RTA Receiver Tube with filtrate and place the RTA Spin Filter into a new RTA Receiver Tube.

4. Second Washing of the RTA Spin Filter
Add 700 µl **Wash Buffer R2** to the RTA Spin Filter and centrifuge at 11.000 x g (11.000 rpm) for 1 minute.
Discard the RTA Receiver Tube with filtrate and place the RTA Spin Filter into a new RTA Receiver Tube.
Remove the residual ethanol by final centrifugation for 4 minutes at maximum speed.
Discard the RTA Receiver Tube with filtrate.

5. Elution of the DNA/ RNA
Place the Spin Filter into a 1.5 ml Elution Tube and add 60 µl of the **Elution Buffer R** (prewarmed to 65°C) directly onto the RTA Spin Filter surface.
Incubate for 3 minutes at RTand centrifuge at 11.000 x g (11.000 rpm) for 1 minute.

Protocol 2: Simultaneous isolation of total nucleic acids (DNA and RNA) from swab material

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

Important Note: *Prewarm the needed amount of **Elution Buffer R** to 65°C for the final elution step.*

1. Sample Lysis

Place the swab into the provided Extraction Tube and add 400 µl of ddH_2O. Place the Extraction Tube into a thermomixer and incubate under continuously shaking for 15 minutes at 65°C and for 10 minutes at 95°C, which leads to higher sensitivity.

Important Note: *To get maximum yield of viral nucleic acids it is essential to leave the swab during the complete lysis time into the reaction tube. It is possible to cut the shaft of the swab, so that you can close the cap of the Extraction Tube (II). It is also possible to do the lysis step with opened cap. The removing of the swab from the Extraction Tube (II) ahead of time will be lead to a dramatically reduced final yield!*
After lysis time carefully squeeze out the swab on the wall of the tube and discard the swab

2. Binding of the DNA and RNA

Add 400 µl **Binding Solution** to the provided **Extraction Tube** and mix the sample completely by pipetting up and down or by vortexing. Transfer the sample into the RTA Spin Filter Set. Close the cap and centrifuge for 2 minutes at 11.000 x g (11.000 rpm). Discard the RTA Receiver Tube with filtrate and place the RTA Spin Filter into a new RTA Receiver Tube.

3. First Washing of the RTA Spin Filter

Add 500 µl **Wash Buffer R1** to the RTA Spin Filter and centrifuges at 11.000 x g (11.000 rpm) for 1 minute.
Discard the RTA Receiver Tube with filtrate and place the RTA Spin Filter into a new RTA Receiver Tube.

4. Second Washing of the RTA Spin Filter

Add 700 µl **Wash Buffer R2** to the RTA Spin Filter and centrifuge at 11.000 x g (11.000 rpm) for 1 minute.
Discard the RTA Receiver Tube with filtrate and place the RTA Spin Filter into a new RTA Receiver Tube.
Remove the residual ethanol by final centrifugation for 4 minutes at maximum speed.
Discard the RTA Receiver Tube with filtrate.

5. Elution of the DNA/ RNA

Place the RTA Spin Filter into a 1.5 ml Elution Tube**.** Add 60 µl of the **Elution Buffer R** (prewarmed to 65°C) directly onto the RTA Spin Filter surface. Incubate for 3 minutes at RT and centrifuge at 11.000 x g (11.000 rpm)for 1 minute.

Protocol 3: Simultaneous isolation of total nucleic acids (DNA and RNA) from cell culture supernatants

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

Important Note: *Prewarm the needed amount of **Elution Buffer R** to 65°C for the final elution step.*

1. Sample Lysis

Mix 200 µl of the cell culture supernatant (cell culture media) with 200 µl of ddH_2O.
Transfer the sample into the provided Extraction Tube. Close the cap and vortex shortly.
Place the Extraction Tube into a thermomixer and incubate under continuously shaking for 15 minutes at 65°C and for 10 minutes at 95°C, which leads to higher sensitivity.

2. Binding of the DNA and RNA

Add 400 µl **Binding Solution** to the provided **Extraction Tube** and mix the sample completely by pipetting up and down or by vortexing. Transfer the sample into the Spin Filter Set. Close the cap and centrifuge for 2 minutes at 11.000 x g (11.000 rpm). Discard the RTA Receiver Tube with filtrate and place the RTA Spin Filter into a new RTA Receiver Tube.

3. First Washing of the RTA Spin Filter

Add 500 µl **Wash Buffer R1** to the RTA Spin Filter and centrifuges at 11.000 x g (11.000 rpm) for 1 minute.
Discard the RTA Receiver Tube with filtrate and place the RTA Spin Filter into a new RTA Receiver Tube.

4. Second Washing of the RTA Spin Filter

Add 700 µl **Wash Buffer R2** to the RTA Spin Filter and centrifuge at 11.000 x g (11.000 rpm). for 1 minute.
Discard the RTA Receiver Tube with filtrate and place the RTA Spin Filter into a new RTA Receiver Tube.
Remove the residual ethanol by final centrifugation for 4 minutes at maximum speed.
Discard the RTA Receiver Tube with filtrate.

5. Elution of the DNA/ RNA

Place the RTA Spin Filter into a 1.5 ml Elution Tube**.** Add 60 µl of the **Elution Buffer R** (prewarmed to 65°C) directly onto the RTA Spin Filter surface. Incubate for 3 minutes at RT and centrifuge at 11.000 x g (11.000 rpm) for 1 minute.

Protocol 4: Simultaneous isolation of total nucleic acids (DNA and RNA) 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 R*** *to 65°C for the final elution step.*

1. Sample Lysis

Transfer 1 - 10 mg of the tissue sample into the provided Extraction Tube. Add 400 µl of ddH_2O. Close the cap and vortex shortly. Place the provided Extraction Tube into a thermomixer and incubate under continuously shaking for 15 minutes at 65°C and for 10 minutes at 95°C, which leads to higher sensitivity.
Lysis time at 65°C can be increased up to 30 minutes.

Important: *A longer lysis time could reduce the final yield and the 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 reaction tube (not provided).

2. Binding of the DNA and RNA

Add 400 µl **Binding Solution** to the 1.5 ml reaction tube and mix the sample completely by pipetting up and down or by vortexing. Transfer the sample into the RTA Spin Filter Set. Close the cap and centrifuge for 2 minutes at 11.000 x g (11.000 rpm). Discard the RTA Receiver Tube with filtrate and place the RTA Spin Filter into a new RTA Receiver Tube.

3. First Washing of the RTA Spin Filter

Add 500 µl **Wash Buffer R1** to the RTA Spin Filter and centrifuges at 11.000 x g (11.000 rpm). for 1 minute.
Discard the RTA Receiver Tube with filtrate and place the RTA Spin Filter into a new RTA Receiver Tube.

4. Second Washing of the RTA Spin Filter

Add 700 µl **Wash Buffer R2** to the RTA Spin Filter and centrifuge at 11.000 x g (11.000 rpm) for 1 minute.
Discard the RTA Receiver Tube with filtrate and place the RTA Spin Filter into a new RTA Receiver Tube.
Remove the residual ethanol by final centrifugation for 4 minutes at maximum speed.
Discard the RTA Receiver Tube with filtrate.

5. Elution of the DNA/ RNA

Place the RTA Spin Filter into a 1.5 ml Elution Tube**.** Add 60 µl of the **Elution Buffer R** (prewarmed to 65°C) directly onto the RTA Spin Filter surface. Incubate for 3 minutes at RT and centrifuge at 11.000 x g (11.000 rpm) for 1 minute.

Protocol 5: Extraction of viral nucleic acids from supernatant of stool suspension

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

Important Note: *Prewarm the needed amount of* ***Elution Buffer R*** *to 65°C for the final elution step.*

1. Sample Lysis

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 (13.000 rpm).
Transfer 400 µl virus containing supernatant into the Extraction Tube (prevent the aspiration of swimming particles).
Place the provided Extraction Tube into a thermomixer and incubate under continuously shaking for 15 minutes at 65°C and for 10 minutes at 95°C, which leads to higher sensitivity.
.
If you want to add nucleic acids for extraction control, please add at this point

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

2. Binding of the DNA and RNA

Add 400 µl **Binding Solution** to the 1.5 ml reaction tube and mix the sample completely by pipetting up and down or by vortexing. Transfer the sample into the RTA Spin Filter Set. Close the cap and centrifuge for 2 minutes at 11.000 x g (11.000 rpm). Discard the RTA Receiver Tube with filtrate and place the RTA Spin Filter into a new RTA Receiver Tube.

3. First Washing of the RTA Spin Filter

Add 500 µl **Wash Buffer R1** to the RTA Spin Filter and centrifuges at 11.000 x g (11.000 rpm). for 1 minute.
Discard the RTA Receiver Tube with filtrate and place the RTA Spin Filter into a new RTA Receiver Tube.

4. Second Washing of the RTA Spin Filter

Add 700 µl **Wash Buffer R2** to the RTA Spin Filter and centrifuge at 11.000 x g (11.000 rpm) for 1 minute.
Discard the RTA Receiver Tube with filtrate and place the RTA Spin Filter into a new RTA Receiver Tube.
Remove the residual ethanol by final centrifugation for 4 minutes at maximum speed.
Discard the RTA Receiver Tube with filtrate.

5. Elution of the DNA/ RNA

Place the RTA Spin Filter into a 1.5 ml Elution Tube**.** Add 60 µl of the **Elution Buffer R** (prewarmed to 65°C) directly onto the RTA Spin Filter surface. Incubate for 3 minutes at RT and centrifuge at 11.000 x g (11.000 rpm) for 1 minute.

Troubleshooting

Problem	Probable cause	Comments and suggestions
clogged RTA Spin-Filter	insufficient lysis, homogenization and/or too much starting material	increase lysis time increase g-force and/ or centrifugation time reduce amount of starting material all centrifugation steps should be conducted at room
low amount of extracted DNA/ RNA	insufficient lysis	increase lysis time
	incomplete elution	reduce amount of starting material. Overloading of RTA Spin Filter reduces yield prolong the incubation time with prewarmed Elution Buffer R to 5 -10 min. Do the elution steps twice Take higher volume of **Elution Buffer R**.
	insufficient mixing of the sample with **Binding Solution**	mix sample sufficient by pipetting up and down with **Binding Solution** prior to transfer the sample onto the Spin Filter RTA
	incomplete removal of cell culture medium	make sure that the cell culture medium is complete removed after the cell harvest
low concentration of extracted DNA/ RNA	too much **Elution Buffer**	elute the DNA/ RNA twice with lower volume of **Elution Buffer R**
	incorrect storage of starting material	ensure that the storage of starting material was correct avoid thawing of the material
DNA/ RNA does not perform well in downstream-applications (e.g. RT-PCR or PCR)	ethanol carryover during elution	increase g-force or centrifugation time when drying the RTA Spin Filter
	salt carryover during elution	ensure that the Wash Buffers are at room temperature check up the **Wash Buffers** for salt precipitates. If there are any precipitates, dissolve these precipitates by carefully warming

Appendix

General notes on handling DNA

Nature of DNA
The length and delicate physical nature of DNA require 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 it will work well in various downstream applications. Damaged DNA could perform poorly in applications such as genomic Southern blotting, long-template PCR, and construction of cosmid libraries.

Handling fresh and stored material before the extraction of DNA
For the isolation of genomic DNA from cells or tissues, use either fresh samples or samples that have been quickly frozen in liquid nitrogen and stored at -70°C. This procedure minimizes degradation of crude DNA by limiting the activity of endogenous nucleases.

Storage of DNA
Store genomic DNA at +2 to +8°C. Storing genomic DNA at -15 to -25°C can cause shearing of DNA, particularly if the DNA is exposed to repeated freeze-thaw cycles. Plasmid DNA and other small circular DNAs can be stored at +2 to +8°C or at -15 to -25°C.

Drying, dissolving and pipetting DNA
Avoid overdrying 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. Plasmid DNA and other small circular DNAs can be vacuum-dried.

To help dissolve the DNA, carefully invert the tubes several times after adding buffer and tap the tube gently on the side. Alternatively let the DNA stand in buffer overnight at +2 to +8°C. Minimize vortexing of genomic DNA since this can cause shearing.

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. Regular pipette tips pose no problem for plasmid DNA and other small.

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 Nucleic Acids as well as DNA or RNA which is co purified.

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 has 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 240°C 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 37°C and then be autoclaved or heated to 100°C 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 plastic ware should be treated before use to ensure that it is RNase free. Plastic ware should be thoroughly rinsed with 0.1 M NaOH, 1 mM EDTA followed by RNase free water. You can also take chloroform-resistant plastic ware rinsed with chloroform to inactivate RNases.
- All buffers must be prepared with 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 *in vitro* diagnostic laboratory practice.

Storage of RNA
Purified RNA can be stored –80°C and is stable for months and years e.g. precipitated and stored in 70% ethanol.

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 Nucleic Acids as well as DNA or RNA, which is co-purified.

Ordering information

Product	Package size	Catalogue No.
RTP® DNA/ RNA Virus Mini Kit	250 preparations	1040100300

Related products

InviMag® Virus RNA Kit/ KF96	5 x 96 preparations	7443300200
InviMag® Virus DNA/RNA Mini Kit/ KFmL	300 preparations	2441150400
Invisorb® Spin Virus DNA Mini Kit	250 preparations	1040200300

Possible suppliers for Isopropanol:

Carl Roth
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Ordering No. 6752

Applichem
2-Propanol für die Molekularbiologie
Ordering No. A3928

Sigma
2-Propanol
Ordering No. 59304-1L-F

Possible suppliers for Centrifuges:

Eppendorf AG
22331 Hamburg, Germany
Phone: +49 (0) 40 53801 0
Fax: +49 (0) 40 53801 556
E-Mail: eppendorf@eppendorf.com
Internet: www.eppendorf.com

SIGMA Laborzentrifugen GmbH
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