INVITEK
Molecular

USER MANUAL

Invisorb® Spin Virus DNA Mini Kit

for viral DNA purification from up to 200 µl serum, plasma, whole blood, cell-free body fluids, cell culture supernatants; rinse liquid from swabs, 1 x 10^6 mammalian cells

CE IVD

REF 1040200300

Invitek Molecular GmbH · D-13125 Berlin

Instruction for the Invisorb® Spin Virus DNA Mini Kit

The **Invisorb® Spin Virus DNA Mini Kit** is the ideal tool using Invisorb® technology for the isolation and purification of high quality viral DNA from DNA viruses contained in up to 200 µl serum, plasma, cerebrospinal fluid, other cell free body fluids and cell culture supernatants, swab material, rinse liquid from swabs), cells ($1x10^6$ mammalian cells) and fresh human whole blood samples 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*, as well as small samples whole blood can be used.

The kit is neither validated for the isolation of viral RNA or total RNA.

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 the Invisorb® Spin Virus DNA Mini Kit

	250 viral DNA extractions
Catalog No.	1040200300
Lysis Buffer HL	60 ml
Carrier RNA	for 3 x 2 ml working solution
RNase Free Water	3 x 2 ml
Binding Buffer HL	2 x 8 ml (final volume 2 x 32 ml)
Proteinase K	for 3 x 2 ml working solution
Wash Buffer I	80 ml (final volume 160 ml)
Wash Buffer II	2 x 60 ml (final volume 2 x 200 ml)
Elution Buffer	30 ml
RTA Spin Filter Set	5 x 50
RTA Receiver Tubes	15 x 50
1.5 ml Receiver Tubes	10 x 50
Manuals	1
Initial steps	Add 24 ml 99.7% Isopropanol to each **Binding Buffer HL.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times. Add 2 ml ddH_2O to **Proteinase K**, mix thoroughly until completely dissolving. Dilute **Carrier RNA** by addition of 2.0 ml **RNase Free Water.** Mix thoroughly until completely dissolving Add 80 ml of 96-100% ethanol to **Wash Buffer I**, mix thoroughly and always keep the bottle firmly closed! Add 140 ml of 96 -100% ethanol to **Wash Buffer II**, 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 **Invisorb® Spin Virus DNA Mini Kit**, except **dissolved Proteinase K** and the **dissolved Carrier RNA** should be stored at room temperature and are stable for at least 12 months.

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).

Proteinase K: Dissolved Proteinase K must be stored at 2 - 8 °C for up to two months. For longer storage –20 °C is recommended, freeze-thaw once only. Therefore, the dissolved Proteinase K is stable as indicated on the kit package.

Carrier RNA: Dissolved Carrier RNA must be stored at -20°C. Therefore, the dissolved mix is stable as indicated on the kit package.

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

Binding Buffer HL charged with isopropanol should be appropriately sealed and stored at room temperature.

Quality control and product warranty

Invitek Molecular warrants the correct function of the **Invisorb® Spin Virus DNA 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 **Invisorb® Spin Virus DNA 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 **Invisorb® Spin Virus DNA 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 **Invisorb® Spin Virus DNA Mini Kit** is the ideal tool for reliable and fast manual isolation and purification of high quality viral DNA from serum, urine, plasma, cerebrospinal fluid, other cell free body fluids and cell culture supernatants, swab material and whole blood. For reproducible high yields an appropriate sample storage and quick operation under the rules for DNA operation is essential. The purified viral DNA is ready to use for *in vitro* diagnostic analysis.

Fresh or frozen whole blood treated with EDTA or citrate, *but not with heparin*, from common blood collection systems can be used.

The isolation protocol and all buffers are optimized to assure a high yield as well as a high purity of viral DNA. All manual work is reduced to a minimum.

THE PRODUCT IS INTENDED 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 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 kit is neither validated for the isolation of viral RNA from serum or plasma, nor from bacteria, fungi, parasites, or purification of total RNA.

The kits applicability for tissue, stool sample, dried blood stains also have not been validated.

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 **Invisorb® Spin Virus DNA Mini Kit** procedures 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 **Invisorb® Spin Virus DNA Mini Kit** to which they apply are listed below as follows:

Lysis Buffer HL:

Warning
H302-315-319 P280-305-351-338

Binding Buffer HL

Danger
H302-H318-P280-P305+P351+P338

Proteinase K:

Danger
H315-319-334-335 P280-P305-P351-P338

Wash Buffer I

Warning
H302-H412-P280-P305-P351-P338-P273-EUH032

H302: Harmful if swallowed.
H315: Causes skin irritation.
H318: Causes serious eye damage.
H319: Causes serious eye irritation.
H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335: May cause respiratory irritation.
H412: Harmful to aquatic life with long lasting effects.
P273: Avoid release to the environment.
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.
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 Invisorb® Spin Virus DNA Mini Kit

starting material	yield	time
1 - 200 µl fresh, frozen or old human or other mammalian whole blood (EDTA, Citrate); up to 200 µl cell free body fluids (plasma, serum etc.); swab material, rinsed liquid from swab; cell culture supernatant from 1 x 10^6 mammalian cells	depends on the sample (storage and source) ***Note:*** *The added Carrier RNA will account for most of the eluted viral DNA. Quantitative RT-PCR is recommended for determination of the viral DNA yield*.	approx. 25 min

The **Invisorb® Spin Virus DNA Mini Kit** provides a fast and efficient way for reliable isolation of high quality viral DNA from DNA viruses found in a diverse range of starting material. The procedure is suitable for use with plasma or serum; either can contain Citrate or EDTA (but no heparine) and other samples. Samples can be fresh, lyophilized or frozen, provided they have not been frozen and thawed more than once. The procedure can be used for isolation of viral DNA from a broad range of DNA viruses. The kit is designed for simultaneous processing of multiple samples.

The amount of purified viral DNA in the **Invisorb® Spin Virus DNA Mini Kit** procedure depends on the sample type, the virus titer, sample source, transport, storage, and age.

The **Invisorb® Spin Virus DNA Mini Kit** simplifies viral DNA isolation by combining efficient lyses of the starting material, the inactivation of DNases and proteins degradation during the lyses with Proteinase K. The liberated DNA is bound onto the membrane of the RTA Spin Filter. Contaminants are removed by repeated wash steps and the purified viral DNA can be eluted in a small volume of Elution Buffer. The isolated viral DNA is ready to use and should be stored at - 80°C.

Yield and quality of isolated viral DNA 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 is ready to use for a broad panel of downstream applications like:

Downstream Application

- Real Time-PCR*, qPCR, PCR
- TaqMan® analysis
- Array technologies

The purification procedure is rapid and requires minimal interaction by the user, allowing safe handling of potentially infectious samples.

Please do not hesitate to contact our technical support **+49 (0)30-9489-2901/ 2907/ 2910 or your local distributor.**

*) The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

Principle and procedure

The **Invisorb® Spin Virus DNA Mini Kit** procedure comprises the following steps:

- lysis of the virus particle
- adjustment of the binding conditions, followed by the transfer of the sample into the DNA binding RTA Spin Filter.
- binding of the viral DNA to the membrane of the RTA Spin Filter.
- washing of the membrane and elimination of contaminants and ethanol
- elution of highly pure viral DNA from the membrane.

Repeated wash steps make sure that contaminations and enzyme inhibitors are efficiently removed and high purified DNA is eluted in **Elution Buffer.**

This manual contains 6 protocols.

Sampling and storage of starting material

Best results are obtained using freshly extracted samples. After collection and centrifugation, serum, plasma, from blood (treated with anticoagulants like EDTA or citrate, *but not with heparin)*, urine, synovial fluid samples or other cell free body fluids, as well as or rinsed liquid from swabs 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 should be avoided.

It leads to denaturation and precipitation of proteins, resulting in reduced viral titers and therefore reduced yields of viral DNA. In addition, cryoprecipitates formed during freeze-thawing can give problems. If cryoprecipitates are visible, they should be pelleted by centrifugation at app. 6800 x g for 3 minutes. The cleared supernatant should be aspirated, without disturbing the pellet and processed immediately.

This step will not reduce viral titers, excluding some few viruses known is e.g. HIV.

Blood and buffy coat

Mammalian blood samples (stabilized with EDTA or citrate) can be stored at room temperature (18-25°C) for 2 - 3 hours, for short time storage (up to 24 h) samples may be stored at 2 - 8 °C. For long-term storage, we recommend freezing samples at – 20°C or – 80°C. Multiple thawing and freezing before isolating the DNA should be avoided. If cryoprecipitate (formed during thawing of frozen samples) are visible avoid aspirating them, they could clog the RTA Spin Filter membrane. Various different primary tubes, blood collection systems (e.g. Sarstedt, Greiner) and anticoagulants (except heparin) can be used to collect blood samples for the Invisorb® procedure.

CSF and bone marrow

Best results are obtained with fresh material. It can be stored for 2-3 h at 4°C,
for longer storage freeze the sample at –20°C. However, often the sample will be dried. The have to be stored cooled at 4°C in a dry surrounding.

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 DNA

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 lysis conditions in the presence of **Proteinase K** and **Lysis Buffer HL** cells are quickly broken and DNases are inactivated simultaneously. The viral DNA is secured. The addition of **Carrier RNA** is necessary for the enhancement of viral DNA recovery so a very small number of viral DNA molecules will also be purified. Carrier RNA also stabilizes nucleic acids in samples with very small nucleic acid concentrations.

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

Removing of residual contaminations
Contaminants are efficiently washed away using **Wash Buffer I** and **Wash Buffer II**, while the viral DNA remains bound to the membrane of the RTA Spin Filter.

Elution
High quality viral DNA is eluted from the membrane using **Elution Buffer**. The eluted viral DNA is ready to use in different subsequent applications.

Important indications

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 and thawing will clog the RTA Spin Filter membrane.

Adding Carrier RNA
Carrier RNA serves two purposes. Firstly, it enhances the binding of viral acids to the RTA Spin Filter membrane, by saturation of non-specific binding sites of the vials 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 DNA degradation in the rare event that DNase molecules are not denaturated by the chaotropic salt and detergents in the Lysis Buffer HL. If Carrier RNA is not added to the Lysis Buffer HL, this may lead to reduced viral DNA recovery.

The use of an internal control is recommended when using the **Invisorb® Spin Virus DNA Mini Kit** in combination with diagnostic amplification systems. Internal Control DNA and reconstituted Carrier RNA should be added to the Lysis Solution HL and mixed thoroughly by inverting the tube 10 times.

Eluting viral DNA
For downstream applications, that require small staring volumes, viral DNA may be eluted in 30 µl Elution Buffer.

The volume of eluate recovered may be less than the volume of elution buffer applied to the RTA Spin Filter.

Handling of RTA Spin Filter
Do to the sensitivity of viral DNA amplification technologies the following precautions are necessary when handling RTA Spin Filter to avoid cross-contamination between sample preparation

- carefully apply the sample or solution to the RTA Spin Filter, pipet the sample into the filter without wetting the rim of the column
- always change pipet tips between liquid transfers, we recommend the use of aerosol-barrier pipet tips
- avoid touching the RTA Spin Filter membrane with the pipet tip

Yield and quality of viral DNA

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 viral DNA.

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 NA.

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 DNA yield.

***** *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.*

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 6). Do not use damaged kit components, since their use may lead to poor kit performance.

- Always change pipet tips between liquid transfers. To avoid cross-contamination, we recommend the use of aerosol-barrier pipet tips.
- All centrifugation steps are carried out at room temperature.
- 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 unless the lot numbers are identical.
- Avoid microbial contamination of the kit reagents.
- To minimize the risk of infections from potentially infectious material, we recommend working under laminar air-flow until the samples are lysed.
- This kit should only be used by trained personal.

Preparing reagents and buffers

1. Adjust the thermomixer to 56°C.
2. Warm up the needed amount of **Elution Buffer** to 56°C.
   (100 - 200 µl **Elution Buffer** are needed per sample).
3. Label the needed amount of Receiver Tubes and RTA Spin Filters.
4. Add the needed µl dd H_2O to reaction tube with **Proteinase K.** Vortex for 5 s.
5. Add the needed amount of ethanol to the **Wash Buffer I** and **II**.

250 viral DNA extractions:
Add 24 ml 99.7% Isopropanol to each **Binding Buffer HL.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times.
Add 2 ml dd H_2O to **Proteinase K**, mix thoroughly until completely dissolving!
Add 2 ml **RNase Free Water** to the **Carrier RNA,** mix thoroughly until completely dissolving
Add 80 ml of 96 - 100 % ethanol to **Wash Buffer I**
Add 140 ml of 96 - 100 % ethanol to **Wash Buffer II**, mix thoroughly and always keep the bottle firmly closed!

Reagents and equipment 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 online in convenient and compact PDF format at www.invitek-molecular.com under each Invitek Molecular kit and kit component.

- Microcentrifuge*
- Thermomixer (for 56°C)
- Measuring cylinder (250 ml)
- Disposable gloves
- Pipette and pipette tips
- Vortexer
- dd H_2O
- 96 - 100 % ethanol
- 1 x PBS (optional)
- Isopropanol**

*The **Invisorb® Spin Virus DNA 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

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

***Possible suppliers:**

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

SIGMA Laborzentrifugen GmbH
37507 Osterode am Harz, Germany
Tel.: +49-5522-5007-0
Fax: +49-5522-5007-12
E-Mail: info@sigma-zentrifugen.de
Internet: www.sigma-zentrifugen.de

Important indications

1. Process only as much blood or serum samples as the microcentrifuge allows to process.
2. Blood, serum or plasma sample and buffers should be thoroughly mixed and should have room temperature (RT).
3. The elution can be done by using lower amount of **Elution Buffer**. This may result in a higher concentration of viral DNA. However, pay attention that minimum volume for elution is 30 µl, but this may reduce the yield. Elution volume between 2 x 30 µl up to 100 µl will realize comparable results.
4. The eluted DNA volume can be lower than the added **Elution Buffer** volume. **Elution Buffer** should be preheated to 56 °C.
5. The **Elution Buffer** does not contain EDTA.
6. The yield can be increased, if the incubation time with preheated **Elution Buffer** will be prolonged.
7. Old blood samples often contains coagulates, if coagulates or cryoprecipitate (formed during thawing of frozen samples) are visible avoid aspirating them, they could clog the RTA Spin Filter membrane.

Internal control (IC) / Extraction control

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

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

Instructions

The following notes are valid for all protocols:

Note: *The DNA can also be eluted with a lower volume of Elution Buffer depend on the expected yield of viral DNA). However, pay attention that minimum volume for the elution is **30 µl and that this volume can reduce the maximum yield**.*

Important *After extraction, place the Elution Tube on ice. For long time storage, keep the nucleic acids at –20°C.*

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

Scheme of the Invisorb® Spin Virus DNA Mini Kit

viral DNA

Please read protocols prior the start of the preparation carefully

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Transfer max. 200 µl sample into a 1.5 ml Receiver Tube

Add 200 µl **Lysis Buffer HL** to the sample and mix 5 times by pipetting
Incubate for 5 min at RT while continuously shaking
Add 20 µl **Proteinase K** and 20 µl **Carrier RNA**
Vortex 10 sec

Incubate for 15 min at 56°C while continuously shaking on the thermomixer

Add the Internal Control

Add 200 µl **Binding Buffer HL** *(follow preparing instructions)* and mix by pipetting up and down five times

Take a RTA Spin Filter Set
Transfer lysate onto RTA Spin Filter,
Incubate for 1 min
Centrifuge for 2 min at 11.000 x g (11.000 rpm)
Discard the filtrate and the RTA Receiver Tube
Transfer the RTA Spin Filter in a new RTA Receiver Tube

Add 500 µl **Wash Buffer I**
Centrifuge for 1 min at 11.000 x g (11.000 rpm)
Discard the filtrate and the RTA Receiver Tube
Transfer the RTA Spin Filter in a new RTA Receiver Tube

Add 700 µl **Wash Buffer II**
Centrifuge for 1 min at 11.000 x g (11.000 rpm)
Discard the filtrate
Put the RTA Spin Filter back to the same RTA Receiver Tube
repeat the step once

Discard the filtrate and the RTA Receiver Tube
Transfer the RTA Spin Filter in a new RTA Receiver Tube

Centrifuge for 4 min at maximum speed for ethanol removal
Discard the filtrate and the RTA Receiver Tube

Place the RTA Spin Filter into a 1.5 ml Receiver Tube
Add 100 µl of **Elution Buffer** (preheated to 56°C)
Incubate for 1 min at room temperature
Centrifuge for 1 min at 11.000 x g (11.000 rpm)
Discard the RTA Spin Filter

Close the 1.5 ml Receiver Tube and store the viral DNA sample at 4 °C

Protocol 1: Viral DNA isolation from 1 - 200 µl human and mammalian whole blood, serum or plasma

Whole blood, plasma or serum from blood treated with anticoagulants like EDTA or citrate, but not with heparin, as well as small samples whole blood can be used.

Please read the instructions carefully and conduct the prepared procedure.

Attention: *Please be aware, that you have to prepare the* ***Binding Buffer HL*** *(see instruction page: 11)*

Important *Transfer the needed amount of Elution Buffer into a 2.0 ml Receiver Tube (not included in the kit) and place the tube at 56°C.*

1. Transfer 1 - 200 µl whole blood, serum or plasma into a 1.5 ml Receiver Tube. If sample volume is lower than 200 µl, equilibrate with 1 x PBS Buffer or distilled water to 200 µl.

2. Add 200 µl **Lysis Buffer HL,** mix completely by pipetting up and down (5 times) and incubate for 5 min at RT while continuously shaking.
Add 20 µl **Proteinase K** and 20 µl **Carrier RNA.** Close the cap and mix by vortexing

3. Incubate the reaction tube for 15 min at 56°C while continuously shaking on a thermomixer.

Note*: If you should use a water bath, please vortex the sample during lysis 2 – 5 times.*

Add the Internal Control

4. Add 200 µl **Binding Buffer HL** and mix the sample by pipetting up and down for 4- 5 times. Take a RTA Spin Filter Set. Transfer the mixture into the RTA Spin Filter. Close the RTA Spin Filter and incubate for 1 min at RT.

5. Centrifuge for 2 min at 11.000 x g (11.000 rpm). Discard the filtrate and the RTA Receiver Tube. Place the RTA Spin Filter in a new RTA Receiver Tube.

6. Add 500 µl **Wash Buffer I** to the RTA Spin Filter. Close the RTA Spin Filter.
Centrifuge for 1 min at 11.000 x g (11.000 rpm). Discard the filtrate and the RTA Receiver Tube. Place the RTA Spin Filter in a new RTA Receiver Tube.

7. Add 700 µl **Wash Buffer II** and centrifuge for 1 min at 11.000 x g (11.000 rpm).
Discard the filtrate and put the RTA Spin Filter back in the RTA Receiver Tube.

8. Add 700 µl **Wash Buffer II** and centrifuge for 1 min at 11.000 x g (11.000 rpm).
Discard the filtrate and the RTA Receiver Tube. Place the RTA Spin Filter in a new RTA Receiver Tube.

9. Centrifuge for 4 min at maximum speed to eliminate the ethanol. Discard the filtrate and the RTA Receiver Tube

10. Place the RTA Spin Filter in a new 1.5 ml Receiver Tube. Add 100 µl of the preheated (56°C) **Elution Buffer** onto the center of the RTA Spin Filter. Incubate at room temperature for 1 min.

11. Centrifuge at 11.000 x g (11.000 rpm) for 1min. Discard the RTA Spin Filter.
Close the lid of the 1.5 ml Receiver Tube and store the viral DNA at 4°C.

Protocol 2: Viral DNA isolation from non-mammalian blood sample material

If you want to use bird (e.g. chicken) or fish blood that contain nucleated erythrocytes, the use of only 10-15 µl of starting material is recommended.

Please read the instructions carefully and conduct the prepared procedure.

Attention: *Please be aware, that you have to prepare the* ***Binding Buffer HL*** *(see instruction page: 11)*

Important *Transfer the needed amount of Elution Buffer into a 2.0 ml Receiver Tube (not included in the kit) and place the tube at 56°C.*

1. Transfer 1 - 25 µl whole blood into a 1.5 ml Receiver Tube and equilibrate with 1 x PBS Buffer to 200 µl.

2. Add 200 µl **Lysis Buffer HL,** mix completely by pipetting up and down (5 times) and incubate for 5 min at RT while continuously shaking.
Add 20 µl **Proteinase K** and 20 µl **Carrier RNA.** Close the cap and mix by vortexing.

3. Incubate the reaction tube for 15 min at 56°C while continuously shaking on a thermomixer.

Note*: If you should use a water bath, please vortex the sample during lysis 2 – 5 times.*

Add the Internal Control

4. Add 200 µl **Binding Buffer HL** and mix the sample by pipetting up and down for 4 - 5 times. Take a RTA Spin Filter Set. Transfer the mixture into the RTA Spin Filter. Close the RTA Spin Filter and incubate for 1 min at RT.

5. Centrifuge for 2 min at 11.000 x g (11.000 rpm). Discard the filtrate and the RTA Receiver Tube. Place the RTA Spin Filter in a new RTA Receiver Tube.

6. Add 500 µl **Wash Buffer I** to the RTA Spin Filter. Close the RTA Spin Filter.
Centrifuge for 1 min at 11.000 x g (11.000 rpm). Discard the filtrate and the RTA Receiver Tube. Place the RTA Spin Filter in a new RTA Receiver Tube.

7. Add 700 µl **Wash Buffer II** and centrifuge for 1 min at 11.000 x g (11.000 rpm).
Discard the filtrate and put the RTA Spin Filter back in the RTA Receiver Tube.

8. Add 700 µl **Wash Buffer II** and centrifuge for 1 min at 11.000 x g (11.000 rpm).
Discard the filtrate and the RTA Receiver Tube. Place the RTA Spin Filter in a new RTA Receiver Tube.

9. Centrifuge for 4 min at maximum speed to eliminate the ethanol. Discard the filtrate and the RTA Receiver Tube

10. Place the RTA Spin Filter in a new 1.5 ml Receiver Tube. Add 100 µl of the preheated (56°C) **Elution Buffer** onto the center of the RTA Spin Filter. Incubate at room temperature for 1 min.

11. Centrifuge at 11.000 x g (11.000 rpm) for 1 min. Discard the RTA Spin Filter.
Close the lid of the 1.5 ml Receiver Tube and store the viral DNA at 4°C or at –20°C (long time).

Protocol 3: Viral DNA isolation from CFS and bone marrow

Please read the instructions carefully and conduct the prepared procedure!

Attention: *Please be aware, that you have to prepare the* ***Binding Buffer HL*** *(see instruction page: 11)*

Important *Transfer the needed amount of Elution Buffer into a 2.0 ml Receiver Tube (not included in the kit) and place the tube at 56°C.*

Preparation of the starting material:

Fresh material:

- use 1 – 200 µl fresh cerebrospinal fluid
- or 1 - 20 µl bone marrow

Dried material (for example on hematological slides):

- Moisten the dried material with a drop of PBS.
- Add 180 µl PBS to a 1.5 ml Receiver Tube.
- Scrape cytological material into the Receiver Tube using the edge of a clean slide.
- Dissolve the resulting sludge by pipetting up and down.

I. Sample Lysis

1. Transfer the starting material into a 1.5 ml Receiver Tube. If the sample volume is lower than 200 µl, equilibrate with 1 x PBS Buffer or distilled water.

2. Add 200 µl **Lysis Buffer HL,** mix completely by pipetting up and down (5 times) and incubate for 5 min at RT while continuously shaking.

Add 20 µl **Proteinase K** and 20 µl **Carrier RNA.** Close the cap and mix by vortexing.

Important Note: *Now vortex the sample for 10 sec! An incomplete mixing will reduce quality and yield of the isolated DNA.*

3. Incubate the reaction tube for 15 min at 56°C while continuously shaking on a thermomixer.

Note*:* *If you should use a water bath, please vortex the sample during lysis 2 – 5 times.*

Add the Internal Control

Proceed as described in protocol 1 steps 4 – 11.

Protocol 4: Viral DNA isolation from mouth brushes (swabs)

Please read the instructions carefully and conduct the prepared procedure!

Attention: *Please be aware, that you have to prepare the* ***Binding Buffer HL*** *(see instruction page: 11)*

Important *Transfer the needed amount of Elution Buffer into a 2.0 ml Receiver Tube (not included in the kit) and place the tube at 56°C.*

1. Add 200 µl **ddH_2O or rinsed liquid (PBS) from swabs,** 200 µl **Lysis Buffer HL** to a 1.5 ml Receiver Tube. Put the mouth brush (swab) inside. You may cut the mouth brush and close the vial. Mix by vortexing.
Incubate for 5 min at RT.
Add 20 µl **Proteinase K** and 20 µl **Carrier RNA** to the sample. Close the cap and mix by vortexing

2. Incubate the reaction tube for 20 min at 56°C while continuously shaking on a thermomixer.

Note*:* *If you should use a water bath, please mix the sample during lysis 2 – 5 times.*

Add the Internal Control

3. Take out the mouth brush (Swab) by a pincer and squeeze it against the wall of the tube to yield lysis mixture. Then proceed as described in protocol 1 steps 4 – 11.

Protocol 5: Viral DNA isolation from cell culture supernatants

Please read the instructions carefully and conduct the prepared procedure.

Attention: *Please be aware, that you have to prepare the* ***Binding Buffer HL*** *(see instruction page: 11)*

Important *Transfer the needed amount of Elution Buffer into a 2.0 ml Receiver Tube (not included in the kit) and place the tube at 56°C.*

1. Transfer 200 µl of the cell culture supernatant (cell culture media) into a 2.0 ml Receiver Tube. Add 200 µl **Lysis Buffer HL,** mix completely by pipetting up and down (5 times) and incubate for 5 min at RT while continuously shaking.

2. Add 20 µl **Proteinase K** and 20 µl **Carrier RNA.** Close the cap and mix by vortexing

3. Incubate the reaction tube for 15 min at 56°C while continuously shaking on a thermomixer.

Note*: If you should use a water bath, please vortex the sample during lysis 2 – 5 times.*

Add the Internal Control

4. Add 200 µl **Binding Buffer HL** and mix the sample by pipetting up and down for 4 - 5 times. Take a RTA Spin Filter Set. Transfer the mixture into the RTA Spin Filter. Close the RTA Spin Filter and incubate for 1 min at RT.

5. Centrifuge for 2 min at 11.000 x g (11.000 rpm). Discard the filtrate and the RTA Receiver Tube. Place the RTA Spin Filter in a new RTA Receiver Tube.

6. Add 500 µl **Wash Buffer I** to the RTA Spin Filter. Close the RTA Spin Filter.
Centrifuge for 1 min at 11.000 x g (11.000 rpm). Discard the filtrate and the RTA Receiver Tube. Place the RTA Spin Filter in a new RTA Receiver Tube.

7. Add 700 µl **Wash Buffer II** and centrifuge for 1 min at 9.300 x g (10.000 rpm).
Discard the filtrate and put the RTA Spin Filter back in the RTA Receiver Tube.

8. Add 700 µl **Wash Buffer II** and centrifuge for 1 min at 11.000 x g (11.000 rpm).
Discard the filtrate and the RTA Receiver Tube. Place the RTA Spin Filter in a new RTA Receiver Tube.

9. Centrifuge for 4 min at maximum speed to eliminate the ethanol. Discard the filtrate and the RTA Receiver Tube

10. Place the RTA Spin Filter in a new 1.5 ml Receiver Tube. Add 100 µl of the preheated (56°C) **Elution Buffer** onto the center of the RTA Spin Filter. Incubate at room temperature for 1 min.

11. Centrifuge at 11.000 x g (11.000 rpm) for 1 min. Discard the RTA Spin Filter. Close the lid of the 1.5 ml Receiver Tube and store the viral DNA at 4°C.

Protocol 6: Viral DNA isolation from 1 x 10^6 mammalian cells

Please read the instructions carefully and conduct the prepared procedure.

Attention: *Please be aware, that you have to prepare the* ***Binding Buffer HL*** *(see instruction page: 11)*

Important *Transfer the needed amount of Elution Buffer into a 2.0 ml Receiver Tube (not included in the kit) and place the tube at 56°C.*

Harvesting up to 1x10^6 mammalian cells

Cells grown in suspension: Spin up to 1 x 10^6 cells for 5 min at 1,500 rpm. Discard the supernatant and remove all media completely.

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

Important: *Incomplete removal of some cell culture media may inhibit the lysis and dilute the lysate, which will affect the binding of DNA to the RTA Spin Filter.*

Disrupt cells by adding Lysis Buffer HL

For pelleted cells: Loosen cell pellet by flicking the tube and add 200 µl **Lysis Buffer HL**, 200 µl ddH_2O, mix by pipetting up and down for 5 times, incubate for 5 min at RT and add 20 µl **Carrier RNA** and 20 µl **Proteinase K**. Close the cap and vortex shortly. No cell clumps should be visible before proceeding with the next step. Pipet the lysed mixture into a 2.0 ml reaction tube (not provided).

For monolayer cells: Add 200 µl **Lysis Buffer HL**, 200 µl ddH_2O, mix by pipetting up and down for 5 times and incubate 5 min at RT. Add 20 µl **Carrier RNA** and 20 µl **Proteinase K** to monolayer cells. Collect cell lysate with a rubber scraper. Mix thoroughly by pipetting up and down. No cell clumps should be visible before proceeding with the next step. Pipet the lysed mixture into 2.0 ml reaction tube (not provided).

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1. Place the tube with the sample into a thermomixer and incubate under continuously shaking for 15 minutes at 65°C.

Note: If you should use a water bath, please vortex the sample during lysis 2 – 5 times.

Add the Internal Control

4. Add 200 µl **Binding Buffer HL** and mix the sample by pipetting up and down for 4- 5 times. Take a RTA Spin Filter Set. Transfer the mixture into the RTA Spin Filter. Close the RTA Spin Filter and incubate for 1 min at RT.

5. Centrifuge for 2 min at 11.000 x g (11.000 rpm). Discard the filtrate and the RTA Receiver Tube. Place the RTA Spin Filter in a new RTA Receiver Tube.

6. Add 500 µl **Wash Buffer I** to the RTA Spin Filter. Close the RTA Spin Filter.
Centrifuge for 1 min at 11.000 x g (11.000 rpm). Discard the filtrate and the RTA Receiver Tube. Place the RTA Spin Filter in a new RTA Receiver Tube.

7. Add 700 µl **Wash Buffer II** and centrifuge for 1 min at 11.000 x g (11.000 rpm).
Discard the filtrate and put the RTA Spin Filter back in the RTA Receiver Tube.
8. Add 700 µl **Wash Buffer II** and centrifuge for 1 min at 11.000 x g (11.000 rpm).
Discard the filtrate and the RTA Receiver Tube. Place the RTA Spin Filter in a new RTA Receiver Tube.

9. Centrifuge for 4 min at maximum speed to eliminate the ethanol completely. Discard the filtrate and the RTA Receiver Tube

10. Place the RTA Spin Filter in a new 1.5 ml Receiver Tube. Add 100 µl of the preheated (56°C) **Elution Buffer** onto the center of the RTA Spin Filter. Incubate at room temperature for 1 min.

11. Centrifuge at 11.000 x g (11.000 rpm) for 1 min. Discard the RTA Spin Filter. Close the lid of the 1.5 ml Receiver Tube and store the viral DNA at 4°C.

Troubleshooting

Problem	Cause	Comments and suggestions
Low amount of DNA	insufficient lysis	increase lysis time with **Lysis Buffer HL**. reduce amount of starting material. continuously shaking improves lysis efficiency.
	inefficient binding of DNA to the membrane	overloading RTA Spin Filter reduces yield - use correct amount of **Binding Buffer HL** - mix sample with **Binding Buffer HL** by pipetting up and down 4-5 times or by vortexing (5 sec) prior to transfer the sample onto the Spin Filter
	incomplete elution	increase incubation time with preheated **Elution Buffe** to 5 - 10 min eluting twice with each 100 µl **Elution Buffer** use higher volume of **Elution Buffer**
	low DNA concentration in the sample	elute the DNA with lower volume of **Elution Buffer**
RTA Spin Filter surface tints yellow	insufficient lysis	see above
	inefficient washing	wash again with **Wash Buffer I** and **II**
	old material	perform isolation as described in protocol 2
Degraded or sheared DNA	incorrect storage of starting material	ensure the sample is harvested and stored as described on page 8 avoid repeated thawing and freezing of the material
	old material	old material often contains degraded DNA Ensure that the starting material is fresh or stored under appropriate condition (for long time storage at -80°C) Avoid thawing and freezing of the material
Problems with subsequent applications (e.g. in PCR)	ethanol in the eluated DNA	verify if the recommended centrifugation time has been adhered to increase centrifugation time for the elimination of ethanol if necessary **Wash Buffer**s should be stored at and used at RT
	salt in the eluat	Check up the **Wash Buffers** for salt precipitates. If there are any precipitates, solve these precipitates by careful warming ensure that the **Wash Buffers** are at RT
Clogged RTA Spin Filter	incorrect storage of starting material	perform isolation as described in protocol 2
	insufficient lysis	increase lysis time with **Lysis Buffer HL** increase centrifugation time and/or speed reduce amount of starting material

Appendix

General notes on handling DNA

Starting material

This kit is designed for extraction of viral DNA from sera, plasma, blood, but even human blood is different between individuals depending on age, health and conditions of life. If you are using blood from animals keep in mind that lysis conditions of blood differs depending on the species. Also, remember that non-mammalian blood contains erythrocytes with nuclei. So for special applications adaptation of starting volumes and lyses time may be recommended.

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, long-template PCR.

Storage of DNA

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.

DNA yield

The amount of purified DNA depends on sample source, transport, storage, and age. Various different primary tubes and anticoagulants (except heparin) can be used to collect blood samples for the **Invisorb®** procedure.

Ordering information

Product	Package size	Catalogue No.
Invisorb® Spin Virus DNA Mini Kit	250 preparations	1040200300

Related products

RTP® Virus DNA/RNA Mini Kit	250 preparations	1040100300
Invisorb® Spin Virus RNA Mini Kit	250 preparations	1040300300

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

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

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