Instructions for use
Invisorb® Spin Universal Kit

CE IVD

REF 1050100200
1050100300

Σ 50 preparations
250 preparations

Invitek Molecular GmbH
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Germany

INVITEK
Molecular

Important notes

Thank you for purchasing the **Invisorb® Spin Universal Kit** from Invitek Molecular.

The product serves the purpose of manual isolation of nucleic acids (genomic DNA, bacterial DNA, viral DNA/RNA) from a variety of clinical samples using Spin Column technology.

WARNING! Improper handling and use for other than the intended purpose can cause danger and damage. Therefore, we ask you to read through these instructions for use and follow them carefully. Always keep them handy. To avoid personal injury, also observe the safety instructions.

All versions of the instructions for use can be found on our website for download or can be requested from us: www.invitek-molecular.com

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Technical Support:

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The kit is in compliance with REGULATION (EU) 2017/746 on in vitro diagnostic medical devices. But it is not for in-vitro diagnostic use in countries where the REGULATION (EU) 2017/746 on in vitro diagnostic medical devices is not recognized.

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1. Safety instructions

Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- When and while working with chemicals, always wear protective clothing, disposable gloves and safety glasses.
- Always change pipette tips between liquid transfers. To avoid cross-contamination, we recommend the use of aerosol-barrier pipette tips.
- Do not reuse any consumables.
- 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 airflow until the samples are lysed.

Before handling chemicals read and understand all applicable safety data sheets (MSDS). These are available online at www.invitek-molecular.com.

Dispose of kit residues and waste fluids in accordance with your country's regulations, again refer to the MSDS. Invitek Molecular has not tested the liquid waste generated by the kit 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 must be handled and disposed of according to local safety regulations.

European Community risk and safety phrases for the components of the **Invisorb® Spin Universal Mini Kit** to which they apply are listed below as follows:

Proteinase K

Danger

H315-H319-H334-H335-P280-P305+P351+P338

Lysis Buffer HLT

Warning

H302-H315-H319-P280-P305+P351+P338

H302: Harmful if swallowed.
H315: Causes skin irritation.
H319: Causes serious eye irritation.
H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335: May cause respiratory irritation.
P280: Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing

Emergency medical information can be obtained 24 hours a day from infotrac, www.infotrac.net:

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

2. Product information

2.1 Kit contents

	50 purifications	250 purifications
Catalogue No.	1050100200	1050100300
Lysis Buffer HLT	15 ml/bottle	60 ml/bottle
Proteinase K	1 vial for 1.1 ml working solution	3 vials for 3 x 2 ml working solution
Carrier RNA	1 vial for 1.2 ml working solution	3 vials for 3 x 2 ml working solution
RNase Free Water	2 x 2 ml/vial	15 ml/bottle
Binding Solution (fill with 99.7% Isopropanol)	empty bottle (final volume 15 ml)	empty bottle (final volume 80 ml)
Wash Buffer HLT	30 ml/bottle (final Volume 50 ml)	105 ml/bottle (final Volume 175 ml)
Wash Buffer	2 x 18 ml/bottle (final Volume 2 x 60 ml)	2 x 60 ml/bottle (final Volume 2 x 200 ml)
Elution Buffer M	30 ml/bottle	120 ml/bottle
RTA Spin Filter Set	50 pieces	5 x 50 pieces
RTA Receiver Tubes	2 x 50 pieces	10 x 50 pieces
1.5 ml Receiver Tubes	50 pieces	5 x 50 pieces
2.0 ml Safe–Lock-Tubes	50 pieces	5 x 50 pieces
Short Protocol	1 leaflet	1 leaflet

2.2 Reagents and equipment to be supplied by user

Lab equipment:

- Microcentrifuge (*all protocols were validated with a Centrifuge 5415 D Eppendorf)*
- Optional: centrifuge for 15 or 50 ml
- Thermo shaker (37°C - 95°C)
- Measuring cylinder (250 ml)
- Disposable gloves
- Pipette and pipette tips
- Vortex mixer
- Reaction tubes (1.5 ml, 2.0 ml)

Liquids and solvents:

- DNase/RNase free water or 1 x PBS to adjust sample volume
- 96 - 100 % ethanol (non-denatured)
- Isopropanol*
- Optional (for respiratory samples with high viscosity): saturated acetylcysteine (ACC) solution (200 mg/ml)
- Optional: Lysozyme (10 mg/ml)

*The 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

2.3 Storage, appearance and shelf life

Shelf life: All buffers and kit components should be stored at room temperature and have a shelf life as indicated on the outer kit package label.

After opening, individual components of the kit, as well as components prepared accordingly before first use, have a shelf life of 3 months.

Before each use, make sure that all components are at room temperature. If there are temperature-related precipitates in the solutions, dissolve them by carefully warming (up to 30°C).

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

Wash Buffer: after adding ethanol, it should be firmly closed and stored at room temperature.

Wash Buffer HLT and **Binding Solution:** after adding isopropanol, they should be firmly closed and stored at room temperature.

Carrier RNA: once dissolved in DNase/RNase free water Carrier RNA must be stored at -20°C.

Proteinase K: once dissolved in DNase/RNase free water Proteinase K can be stored at 2 - 8 °C for up to two months. For longer storage keep at –20 °C, freeze-thaw once only.

2.4 Intended use

The **Invisorb® Spin Universal Kit** is a Spin Column technology based nucleic acid extraction kit, intended for the simultaneous isolation and purification of genomic DNA, bacterial DNA and viral DNA/RNA.

The kit can be used for a variety of human sample types, such as fresh or frozen venous whole blood anticoagulated with EDTA or citrate or the respective plasma preparations, serum, rinsed liquid from swabs, pretreated sputum, BAL, tracheal secrete, cultivated bacteria, supernatant from stool suspension, cerebrospinal fluid, cell culture supernatants, biopsy material/tissue, urine, and other cell-free body fluids.

The product is not intended to be used with heparinized blood samples. The product is intended for use by professionals only, such as laboratory technicians, physicians and biologists trained in molecular biological techniques and *in vitro* diagnostic procedures.

2.5 Product information and specifications

Starting material	Yield	Quality	Time
Up to 200 µl • Serum, plasma, other cell-free body liquids, urine • swabs (dry, stabilized) • supernatant from stool suspensions • cultivated bacteria • tracheal secrete, BAL, sputum • cell culture supernatant Up to 100 µl: • fresh or frozen blood (EDTA / citrate stabilized, but not heparin) Up to 10 mg tissue sample	Depending on sample (storage and source) Whole blood: in average 1 µg DNA	genomic DNA from Blood: A_{260} : A_{280} 1.8 – 2.1 Other sample types: depending on sample type, target nucleic acids	approx. 30 min for 12 samples (excl. lysis)

Yield and quality of purified nucleic acids depend on the sample type, sample source, transport, storage, age, the virus titer and for blood samples also on the leukocyte count.

For determination of yield please note that nucleic acids purified with this kit contain Carrier DNA (5 µg per 200 µl sample), which account for most of the nucleic acids present in the eluate. Especially viral nucleic acids from biological sample material are usually very low concentrated and therefore almost impossible to be quantified photometrically. Quantitative RT-PCR is recommended for yield determination.

The **Invisorb® Spin Universal Kit** provides an efficient procedure for isolation of high-quality nucleic acids. The kit is designed for simultaneous isolation of viral DNA/RNA, bacterial DNA and genomic DNA via a lyse-bind-wash-elute Spin Column protocol.

The kit is validated for leukocyte counts of $3x10^6$ - $1x10^7$ cells/ml. Excessively high cell counts may lead to clogging of the RTA Spin Filter and thus to undesirable effects on the purification process. It is therefore recommended to consider sample input volume as a parameter during the implementation of your *in vitro* diagnostic protocol. If required, samples may be pre-diluted with PBS or DNase/RNase free water prior to the isolation and purification process.

Downstream Applications:
Yield and quality of isolated nucleic acids are in general suitable for plenty of molecular-diagnostic applications such as PCR techniques, NGS, hybridization methods and HLA typing. Downstream applications should be performed according to the respective manufacturers' specifications.

2.6 Principle and procedure

1. Lyse samples
Samples are lysed at elevated temperatures. Lysis is performed in the presence of Lysis Buffer HLT, Proteinase K and optionally lysozyme to break bacterial cell walls and to digest proteins.

The addition of Carrier RNA is required for the enhancement and stabilization of viral DNA/RNA recovery and to purify very small amounts of viral nucleic acids.

2. Bind nucleic acids
By adding Binding Solution to the lysate, optimal binding conditions are adjusted. Each lysate is then applied to an RTA Spin Filter and nucleic acids are adsorbed to the membrane.

3. Wash to remove residual contaminations
Contaminants are efficiently washed away using Wash Buffer HLT and Wash Buffer, while nucleic acids remain bound to the membrane.

4. Elute nucleic acids
Nucleic acids are eluted from the RTA Spin Filter using 100 - 200 µl Elution Buffer M.

3. Nucleic acid extraction with the Invisorb® Spin Universal Kit

3.1 Before starting a protocol

When using the kit for the first time make sure all buffers and reagents are prepared as indicated:

Buffer preparations prior first use: 50 preparations
Carrier RNA: Resuspend lyophilized **Carrier RNA** by addition of 1.2 ml **DNase/RNase free Water** to the vial and mix thoroughly until completely dissolving (at least 1 minute).
Proteinase K: Resuspend lyophilized **Proteinase K** by addition of 1.1 ml **DNase/RNase free Water** to the vial, mix thoroughly until completely dissolving.
Binding Solution (empty bottle): Fill 15 ml **99.7% isopropanol** (molecular biology grade) into the bottle, always keep the bottle firmly closed.
Wash Buffer HLT: Add 20 ml of **99.7% isopropanol** to the bottle**.** Mix thoroughly, always keep the bottle firmly closed.
Wash Buffer: Add 42 ml of **96 -100% ethanol** to the bottle. Mix thoroughly, always keep the bottle firmly closed.
Buffer preparations prior first use: 250 preparations
Carrier RNA: Resuspend lyophilized **Carrier RNA** by addition of 1 ml **DNase/RNase free Water** to the vial and mix thoroughly until completely dissolving (at least 1 minute), then add another 1 ml **DNase/RNase free water**.
Proteinase K: Resuspend lyophilized **Proteinase K** by addition of 2 ml **DNase/RNase free Water** to the vial, mix thoroughly until completely dissolving.
Binding Solution (empty bottle): Fill 80 ml **99.7% isopropanol** (molecular biologic grade) into the bottle.
Wash Buffer HLT: Add 70 ml of **99.7% isopropanol** to the bottle**.** Mix thoroughly, always keep the bottle firmly closed.
Wash Buffer: Add 140 ml of **96 -100% ethanol** to the bottle. Mix thoroughly, always keep the bottle firmly closed.

- Adjust the thermo shaker to 65°C.
- Warm up the needed amount of **Elution Buffer M** to 65°C (50 - 200 µl **Elution Buffer M** are needed per sample).
- Determine the number of required reactions including controls and label the needed amount of RTA Spin Filters (lid) and the needed amount of 1.5 ml Receiver Tubes (per sample: 1 Receiver Tube is needed).

Master mix

For easier handling, we recommend preparing a master mix consisting of Lysis Buffer HLT, Proteinase K and, if required, Carrier RNA. When preparing the master mix, it is recommended to prepare a volume that exceeds the total number of reactions by 5 %.

Always prepare the master mix fresh and shortly before use.

Isolating genomic DNA, bacterial DNA and viral DNA/RNA:
Per sample 200 µl Lysis Buffer HLT, 20 µl Proteinase K and 20 µl Carrier RNA are required.

Isolating genomic DNA:
Per sample 220 µl Lysis Buffer HLT and 20 µl Proteinase K are required. The use of Carrier RNA is not required.

Extraction control

Refer to the manufacturer's instructions to determine the optimal amount of extraction control for specific downstream applications.

Low volumes of extraction control (DNA or RNA) must be combined with the provided Carrier RNA in one mixture. The vials with Carrier RNA contain 1.2 ml or 2.0 ml stock solution, depending on the package size. Add the respective amount of extraction control nucleic acid to the Carrier RNA, if a high volume is necessary (> 25% of the total Carrier RNA Volume), replace the appropriate amount of DNase/RNase free Water during dilution of the Carrier RNA.

3.2 Sampling and storage of starting material

For reproducible and high yields, the correct sample storage is essential. Yields may vary depending on factors such as health of the donor, sample age, sample type, transport and storage.

Repeated freeze-thaw cycles of samples should be avoided to prevent nucleic acid degradation. In general, best results are obtained using fresh samples. It is recommended to consider technical guidance such as e.g., CEN/TS and ISO standards on the pre-examination process for molecular diagnostics under IVDR as highlighted in G. Dagher, et al. (https://doi.org/10.1016/j.nbt.2019.05.002).

Serum, plasma, other cell-free body liquids: Serum or plasma derived from venous whole blood (treated with anticoagulants like EDTA or citrate, but not with heparin), synovial fluid samples or other cell-free body fluids can be used for extraction. Whole blood should not be vortexed as to avoid hemolysis. Allow serum tubes to sit for at least 30 min before centrifugation. Follow blood collection system instructions for preparation of serum or plasma. It is recommended to separate plasma/serum through centrifugation within 12 h. Supernatants obtained using systems without gel separator should be transferred to fresh sample tubes. For short-term storage, samples can be kept on ice for 1-2 hours. For up to 24 h samples can be stored at -20°C. For long-term storage, freezing samples in aliquots at –80°C is recommended. Repeated freeze-thaw cycles may negatively affect sample integrity and cause e.g. denaturation/precipitation of proteins, potentially resulting in reduced yield, quality or viral titers. In addition, cryoprecipitates formed during thaw-freeze cycles can cause problems. If cryoprecipitate is visible, centrifuge at 6.800 x *g* for 3 min. The clear supernatant should be used immediately.

Blood: Blood samples (stabilized with EDTA or citrate but not heparinized) can be stored at room temperature for 2-3 hours. For short-term storage (up to 24 h) samples should be stored at 2-8°C. For long-term storage, freezing samples at -20°C or -80°C is recommended.

Swabs:
Dry swabs: prepare the samples as described in the corresponding sample preparation method. Store dry at 4-8°C.

Swabs in stabilization medium: the stabilization liquid can be handled as cell-free body fluid. Please note that some stabilization agents may cause a reduced yield due to incompatibility with chemistry used in the kit. Store according to the manufacturer's requirements.

Stool samples: Samples contain DNases and RNases which can quickly cause DNA and RNA degradation. Therefore, samples should be stored frozen at – 80°C.

Cultivated bacteria: After cultivation bacteria must be pelleted and frozen at -20°C or -80°C for long-term storage. Resuspension is described in the corresponding sample preparation method.

Urine: Depending on bacteria titer and application a starting volume of 15-50 ml urine is recommended. Centrifuge the sample to pellet bacteria and remove the supernatant completely (urea contaminations can inhibit PCR reactions). For some applications fresh urine can be used directly. For long-term storage, freezing samples at -20°C or -80°C is recommended.

Tracheal secrete, BAL, sputum: Samples contain DNases and RNases, which can quickly cause DNA and RNA degradation. Therefore, samples should be stored frozen at – 80°C.

Tissue Biopsies*:* Samples must be immediately frozen and stored at –20°C or –80°C. Repeated freezing and thawing must be avoided. The amount of purified DNA depends on the type of starting material. Thaw the sample in lysis mixture.

Cell culture supernatants: Prepare supernatant samples like other cell-free body fluid samples described in the corresponding sample preparation method. For long-term storage, freezing samples at -20°C or -80°C is recommended.

3.3 Preparation of starting materials

In the following the preparation of the sample lysis for different starting materials is described. Please use 2 ml Safe-Lock Tubes for sample preparation, as these are also required in the subsequent lysis step. After the preparation of starting materials refer to chapter 3.5 "Protocol: Simultaneous isolation of nucleic acids (DNA and RNA) from liquid samples" to follow step 1a) - d) of the protocol to continue, unless stated otherwise.

3.3.1 Serum, plasma, other cell-free body liquids

Always mix the sample well before extraction.
Use 200 µl sample for extraction. If the sample volume is below 200 µl, adjust with PBS Buffer or DNase/RNase free water to a final volume of 200 µl.

3.3.2 Blood

Always mix the sample well before extraction.
Dilute 100 µl fresh or thawed blood with 100 µl DNase/RNase free water.

3.3.3 Swabs

a) Dry Swabs

Rinse the swabs in a suitable vial in the lowest possible volume of PBS or DNase/RNase-free water (for nasopharyngeal swabs about 400 µl, for oral swabs about 600 µl). Squeeze the swab to the inner wall of the vial to obtain as much sample as possible.
Use 200 µl of the rinsed solution for extraction.
Alternatively, swabs can be directly rinsed in a mix of 200 µl Lysis Buffer HLT, 20 µl Proteinase K, 20 µl Carrier DNA (optional for preparation of genomic DNA) and 200 µl DNase/RNase free water. Incubate swabs for 5-10 min at RT, mix occasionally. Take care to avoid cross contamination.

b) Swabs in stabilization medium

Use 200 µl of the stabilization solution for extraction.
Some stabilization media may interfere with the lysis reaction (if you have any questions, please refer to the FAQ or contact support).

3.3.4 Stool samples (supernatant)

a) Extraction of nucleic acids from viruses

To prepare supernatant transfer 100 µl / 100 mg stool sample into a 2 ml vial and add 900 µl DNase/RNase free water. Vortex for 30 s followed by a 1 min centrifugation step at 12.000 x g. Transfer 200 µl supernatant to a fresh vial for sample extraction. Avoid solid particles in the sample.

b) Extraction of bacterial DNA

To prepare supernatant transfer 100 µl / 100 mg stool sample into a 2 ml vial and add 300 µl DNase/RNase free water. Vortex for 30 s followed by a 30 s centrifugation step at 1.000 x g.

Transfer 200 µl supernatant to a fresh vial for sample extraction. Avoid solid particles in the sample.

3.3.5 Cultivated bacteria

Transfer 1ml of a bacterial overnight culture into a 2.0 ml Safe-Lock-Tube. Centrifuge for 2 min at 10.000 x g and remove the supernatant completely. Resuspend the pellet in 200 µl PBS Buffer and start sample extraction.

3.3.6 Urine

Depending on bacteria titre and application a starting volume of 15-50 ml urine is recommended. Centrifuge the sample to pellet bacteria and remove the supernatant completely (urea contaminations can inhibit PCR reactions). Resuspend the bacteria pellet in 200 µl PBS Buffer.
For some applications 200 µl of fresh urine can be used directly.

3.3.7 Tracheal secrete, BAL, sputum

a) Non-viscous or low viscosity samples

Always mix the sample well before extraction.
Use 200 µl sample for extraction. If the sample volume is below 200 µl, adjust with PBS Buffer or DNase/RNase free water to a final volume of 200 µl.

b) Isolation of bacterial DNA from viscous samples

Transfer 150 µl of the sputum sample or 1 ml tracheal secrete or BAL into a Safe- Lock-Tube and add 150 µl or 1 ml saturated acetylcysteine (ACC) solution respectively (ratio sample to buffer must be 1:1).
Incubate for 10 min at 95°C while continuously shaking.
Centrifuge at 10.000 x g for 5 min. Discard the supernatant.
Resuspend the bacterial pellet in 200 µl PBS or DNase/RNase free water and proceed with the sample extraction.

c) Isolation of viral DNA/RNA from viscous samples

Transfer 150 µl of the sample into a Safe Lock Tube and add 150 µl saturated acetylcysteine (ACC) solution to the sample (ratio sample to buffer must be 1:1).
Incubate for 10 min at 95°C while continuously shaking.
Allow the sample to cool down.
Use 200 µl sample for extraction.

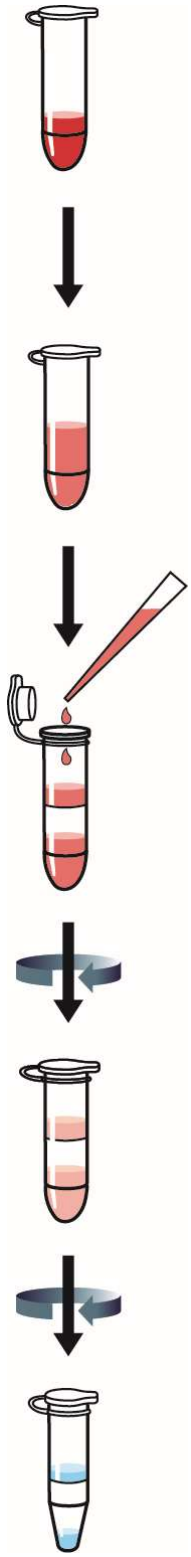
3.3.8 Tissue Biopsies

Transfer 1 - 10 mg tissue biopsy sample into a 2.0 ml Safe-Lock-Tube and add 200 µl DNase/RNase free water or PBS, 200 µl Lysis Buffer HLT, 20 µl Carrier RNA (optional, for low DNA/RNA samples) and 20 µl Proteinase K to each sample.
For disruption of difficult to lyse tissue like cartilage, kidney, and heart muscle: bead beating with Zirconia beads (available separately) is recommended.
After mechanical treatment incubate for 10 min at 65°C while continuously shaking.
Incubate for 10 min at 95°C while continuously shaking.
Centrifuge for 1 minute at 10.000 x g and transfer supernatant to a new tube.
Continue with the extraction protocol at step 2, adding Binding solution.

3.3.9 Cell culture supernatants

Use 200 µl sample for extraction.

3.4 Short protocol Invisorb® Spin Universal Kit



Lyse samples

Refer to chapter 3.3 “Preparation of starting material” for sample specific pre-treatment.

1.a) Purification of bacterial nucleic acids

Mix 200 µl sample* with 20 µl Lysozyme in a 2.0 ml Safe-Lock tube
Incubate 10 min at 37°C
Add 20 µl Carrier RNA, 200 µl **Lysis Buffer HLT** and 20 µl **Proteinase K**
Incubate 15 min at 65°C while shaking

1.b) Simultaneous purification of bacterial and viral nucleic acids

Mix 200 µl sample* with 20 µl Lysozyme in a 2.0 ml Safe-Lock tube
Incubate 10 min at RT
Add 20 µl Carrier RNA, 200 µl **Lysis Buffer HLT** and 20 µl **Proteinase K**
Incubate 10 min at 65°C while shaking
Incubate 10 min at 95°C while shaking

1.c) Purification of viral nucleic acids

Mix 200 µl sample* with 200 µl **Lysis Buffer HLT**, 20 µl **Carrier RNA** and 20 µl **Proteinase K** in a 2.0 ml Safe-Lock tube
Incubate 10 min at 65°C while shaking
Incubate 10 min at 95°C while shaking

1.d) Purification of genomic DNA

Mix 200 µl sample* with 220 µl **Lysis Buffer HLT** and 20 µl **Proteinase K** in a 2.0 ml Safe-Lock tube
Incubate 10 min at 65°C while shaking
Incubate 10 min at 95°C while shaking (skip for blood samples)

*If the sample volume is below 200 µl, adjust with PBS or DNase/RNase free water

Bind nucleic acids

2. Add 260 µl **Binding Solution,** mix by pipetting up and down or vortexing.
   Incubate 5 min at RT
   Transfer the sample into the RTA Spin Filter Set
   Centrifuge 1 min at 11.000 x g
   Discard the RTA Receiver Tube with filtrate and place the RTA Spin Filter into a new RTA Receiver Tube.

Wash to remove residual contaminations

3. Add 600 µl **Wash Buffer HLT,** centrifuge 1 min at 11.000 x g
   Discard the RTA Receiver Tube with filtrate and place the RTA Spin Filter into a new RTA Receiver Tube
4. Add 700 µl **Wash Buffer**, centrifuge 1 min at 11.000 x g
   Discard the filtrate and place the RTA Spin Filter back to the RTA Receiver Tube
5. Repeat this washing step once
6. Centrifuge 5 min at max. speed to remove residual ethanol
   Discard the RTA Receiver Tube with filtrate

Elute nucleic acids

7. Place the Spin Filter into a 1.5 ml Receiver Tube
   Add 50-200 µl **Elution Buffer M** (preheated to 65°C) directly onto the RTA Spin Filter
   Incubate 1 min at RT and centrifuge 1 min at 11.000 x g
   Discard the RTA Spin Filter and store eluted nucleic acids on ice

3.5 Protocol: Simultaneous isolation of nucleic acids (DNA and RNA) from liquid samples

Please refer to chapter 3.3 “Preparation of starting material” for sample specific pre-treatment.

1.a) Sample lysis for purification of bacterial nucleic acids

Mix 200 µl of the sample or resuspended bacterial pellet with 20 µl Lysozyme in a 2 ml Safe-Lock-Tube.
Incubate for 10 min at 37°C
Add 20 µl Carrier RNA. Mix by vortexing.
Add 200 µl **Lysis Buffer HLT** and 20 µl **Proteinase K**.
Alternatively add 240 µl Master Mix to each sample.
Mix thoroughly 10 sec. by vortexing and incubate for 10-15 min at 65°C while continuously shaking.
Optional for hard to lyse bacteria like *Mycobacteria*: incubate for 10 min at 95°C

1.b) Sample lysis for simultaneous purification of bacterial and viral nucleic acids

Mix 200 µl of the sample with 20 µl Lysozyme in a 2 ml Safe-Lock-Tube.
Incubate for 10 min at Room Temperature.
Add 20 µl Carrier RNA. Mix by vortexing.
Add 200 µl **Lysis Buffer HLT** and 20 µl **Proteinase K**.
Alternatively add µl Master Mix to each sample.
Mix thoroughly 10 sec. by vortexing and incubate for 10min at 65°C while continuously shaking.
Incubate for 10 min at 95°C while continuously shaking.

1.c) Sample lysis for purification of viral nucleic acids

Mix 200 µl of the sample with 200 µl **Lysis Buffer HLT**, 20 µl **Carrier RNA** and 20 µl **Proteinase K** in a 2 ml Safe Lock Tube.
Alternatively add 240 µl Master Mix to each sample.
Mix thoroughly 10 sec. by vortexing and incubate for 10-15 min at 65°C while continuously shaking.
Incubate for 10 min at 95°C

1.d) Sample lysis for purification of genomic DNA

Mix 200 µl of the sample with 220 µl **Lysis Buffer HLT** and 20 µl **Proteinase K** in a 2 ml Safe Lock Tube.
Alternatively add 240 µl Master Mix to each sample.
Mix thoroughly 10 sec. by vortexing and incubate for 10-15 min at 65°C while continuously shaking.
Incubate for 10 min at 95°C (skip for isolation of genomic DNA from diluted blood samples)

> ***Note:*** *If you want to add nucleic acids for extraction control, please add them now, before the binding step.*

2. Add 260 µl **Binding Solution** and mix completely by pipetting up and down or by vortexing.
   Incubate the sample at room temperature for 5 minutes.
   Take an RTA Spin Filter Set. Transfer the mixture into the RTA Spin Filter.
   Centrifuge for 1 minute at 11.000 x g.
   Discard the RTA Receiver Tube with filtrate and place the RTA Spin Filter in a new RTA Receiver Tube.
3. Add 600 µl **Wash Buffer HLT** to the RTA Spin Filter and centrifuge 1 min at 11.000 x g.
   Discard the RTA Receiver Tube with filtrate and place the RTA Spin Filter in a new RTA Receiver Tube.
4. Add 700 µl **Wash Buffer** to the RTA Spin Filter and centrifuge 1 min. at 11.000 x g.
   Discard the filtrate and place the RTA Spin Filter in back to the used RTA Receiver Tube.
5. Add 700 µl **Wash Buffer** to the RTA Spin Filter and centrifuge for 1 min. at 11.000 x g.
   Discard the filtrate and place the RTA Spin Filter in back to the used RTA Receiver Tube.
6. Centrifuge for 5 min at 11.000 x g to eliminate the ethanol completely.
   Discard the RTA Receiver Tube with filtrate.
7. Place the RTA Spin Filter in a 1.5 ml Elution Tube.
   Add 50 - 200 µl of the of the preheated (65°C) **Elution Buffer M** directly on the RTA Spin Filter surface.
   Incubate at room temperature for 1 min.
   Centrifuge at 11.000 x g for 1 minute.
   Discard the RTA Spin Filter.
   Close the 1.5 ml Receiver Tube and store the sample at -20 °C to -80°C.

4. Appendix

4.1 Troubleshooting

Problem	Possible cause	Recommendation
Low amount of nucleic acids	Insufficient cell lysis	Increase lysis time with **Lysis Buffer HLT** Continuous shaking improves lysis efficiency Reduce amount of starting material to avoid column overload
	Incomplete elution	Increase incubation time with preheated **Elution Buffer M** to 5-10 min Elute twice with 100 µl **Elution Buffer M** Use a higher volume of **Elution Buffer M**
	Low nucleic acid-concentration in the sample	Elute the nucleic acids with a lower volume of **Elution Buffer M**, do not use volumes lower than 30 µl
	Incorrect storage of starting material	Ensure that starting material is appropriately stored. Avoid repeated thaw-freeze cycles of the sample material.
	Wash Buffers were incorrectly prepared	Ensure, that the correct amount of ethanol/isopropanol is added to the Wash Buffers and that all solutions are stored firmly closed.
	Proteinase K volume/concentration too low	Make sure that the lyophilized Proteinase K is resuspended with the appropriate volume of water before use
Degraded nucleic acids	Incorrect storage of starting material	Ensure the sample is taken and stored correctly, please refer to the FAQ section on our webpage for more information
	Old material	Ensure that the starting material is stored at appropriate conditions (–20°C/-80°C).
Nucleic acids do not perform well in downstream applications (e.g. real-time PCR or NGS)	Ethanol carryover during elution	Increase time of drying step for removal of ethanol.
	Salt carry-over during elution	Check the **Wash Buffers** for salt precipitates. If there are any precipitates visible, solve them by carefully warming up to 30°C Ensure that the **Wash Buffers** are at room temperature before use.
Colored residues on the RTA Spin filter after washing	Insufficient cell lysis	See above
	Inefficient washing	Wash again with **Wash Buffer**
	Wash Buffers were incorrectly prepared	See above

4.2 Warranty

Invitek Molecular guarantees the correct function of the kit for applications described in this manual and in accordance with the intended use. In accordance with Invitek Molecular's EN ISO 13485 certified Quality Management System the performance of all kit components has been tested to ensure product quality.
Any problems, incidents or defects shall be reported to Invitek Molecular immediately upon detection. Immediately upon receipt, inspect the product to ensure that it is complete and intact. In the event of any discrepancies, you must inform Invitek Molecular immediately in writing. Modifications of the kit and protocols and use that deviate from the intended purpose are not covered by any warranty.

Invitek Molecular reserves the right to change, alter, or modify any product to enhance its performance and design at any time.
Invitek Molecular warrants products as set forth in the General Terms and Conditions available at www.invitek-molecular.com. If you have any questions, please contact techsupport@invitek-molecular.com.

4.3 Symbols used on product and labeling

 Manufacturer

 Lot number

 Unique identifier of a medical device

 Catalogue number

 Expiry date

 Consult operating instructions

 Temperature limitation

 Do not reuse

 Amount of sample preparations

 in vitro diagnostic medical device

4.4 Further documents and supplementary information

Visit www.invitek-molecular.com for further information on:

- FAQs and troubleshooting tips
- Manuals in different languages
- Safety data Sheets (MSDS)
- Web support
- Product videos

If, despite careful study of the operating instructions and further information, you still require assistance, please contact us at techsupport@invitek-molecular.com or the dealer responsible for you.

4.5 Ordering information

Product	Package Size	Catalogue No.
Invisorb® Spin Universal Kit	50 preparations	1050100200
Invisorb® Spin Universal Kit	250 preparations	1050100300

Revision history

Revision	Date	Description
EN-v1-2022	2022-05-18	New document

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