Instructions for use
Invisorb® Spin Blood Mini Kit

CE IVD

REF 1031100300

Σ 250 preparations

Invitek Molecular GmbH
Robert-Rössle-Straße 10
13125 Berlin
Germany

INVITEK
Molecular

Important notes

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

The product serves the purpose of isolating genomic DNA from fresh or frozen human blood as well as from buffy coat via a Spin Column based purification procedure.

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

Contact:

Invitek Molecular GmbH

Robert-Rössle-Str. 10, 13125 Berlin, Germany

+ 49 30 9489 2908

www.invitek-molecular.com

Technical Support:

techsupport@invitek-molecular.com

The kit is in compliance with REGULATION (EU) 2017/746 on in vitro diagnostic medical devices. 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.

Table of Contents

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 Blood Mini Kit** to which they apply are listed below as follows:

Proteinase S

Danger

H317-H318-P280-P305+P351+P338

Lysis Buffer HLT

Warning

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

H302: Harmful if swallowed.
H315: Causes skin irritation.
H317: May cause an allergic skin reaction.
H318: Causes serious eye damage.
H319: Causes serious eye 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

	250 purifications
Catalogue No.	1031100300
Lysis Buffer HLT	60 ml/bottle
Binding Solution (fill with 99.7% Isopropanol)	empty bottle (final volume 80 ml)
Elution Buffer M	60 ml/bottle
Proteinase S	3 x 2 ml/vial
Wash Buffer HLT	105 ml/bottle (final volume 175 ml)
Wash Buffer	3 x 45 ml/bottle (final volume 3 x 150 ml)
RTA Spin Filter Set	5 x 50 pieces
2.0 ml Safe–Lock-Tubes	5 x 50 pieces
RTA Receiver Tubes	15 x 50 pieces
1.5 ml Receiver Tubes	5 x 50 pieces
Short Protocol	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)*
- Thermo shaker (for incubation at 56°C)
- Measuring cylinder (250 ml)
- Disposable gloves
- Pipette and pipette tips
- Vortex mixer
- Reaction tubes (1.5 ml or 2.0 ml)

Liquids and solvents:

- DNase/RNase free water or 1 x PBS to adjust sample volume
- 96 - 100 % ethanol (non-denatured)
- Isopropanol*

*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.

Proteinase S is colored blue, making it easier to follow the transfer of small amounts of enzyme.

2.4 Intended use

The **Invisorb® Spin Blood Mini Kit** is a Spin Column technology based nucleic acid extraction kit, intended for the isolation and purification of genomic DNA from human whole blood and buffy coat.

The **Invisorb® Spin Blood Mini Kit** is intended to be used with fresh or frozen venous whole blood anticoagulated with EDTA or citrate and fresh or frozen buffy coat, obtained with common, commercially available blood collection systems.

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
1 - 200 µl fresh or frozen human whole blood (EDTA/citrate stabilized, but not heparinized) 1 – 30 µl buffy coat	up to 10 µg (in average 6 µg) depending on number of leukocytes, sample source, sample transport, sample storage, and age of the sample	A_{260} : A_{280} 1.7 – 2.0	approx. 25 min (incl. lysis)

The **Invisorb® Spin Blood Mini Kit** provides an efficient procedure for isolation of high-quality DNA. The kit is designed for simultaneous processing of multiple samples 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 genomic DNA is 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 and Proteinase S.

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

3. Wash to remove residual contaminations
Contaminants are efficiently washed away using Wash Buffer HLT and Wash Buffer, while the genomic DNA remains bound to the membrane.

4. Elute DNA
Genomic DNA is eluted from the RTA Spin Filter using 30 - 200 µl Elution Buffer M.

3. Nucleic acid extraction with the Invisorb® Spin Blood Mini Kit

3.1 Before starting a protocol

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

Buffer preparations prior first use:
Binding Solution (empty bottle): Fill 80 ml **99.7% isopropanol** (molecular biology grade) into the bottle, always keep the bottle firmly closed. **Wash Buffer HLT:** Add 70 ml of **99.7% isopropanol** to the bottle**.** Mix thoroughly, always keep the bottle firmly closed. **Wash Buffer:** Add 105 ml of **96 -100% ethanol** to the bottle. Mix thoroughly, always keep the bottle firmly closed.

- Adjust the thermo shaker to 56°C
- Warm up the needed amount of **Elution Buffer M** to 56°C
  (30 - 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).

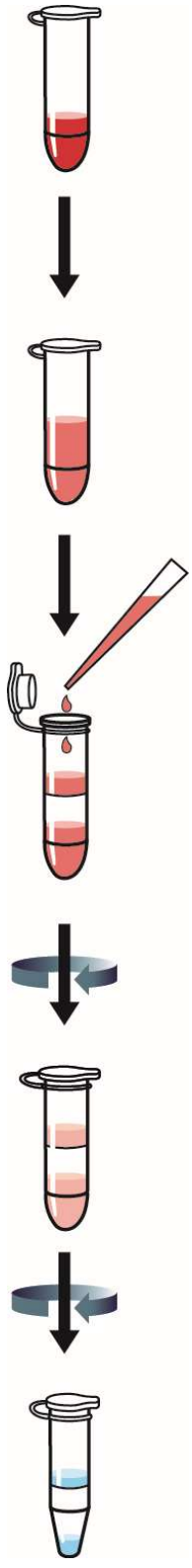
3.2 Sampling and storage of starting material

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.

Human blood samples (stabilized with EDTA or citrate but not heparin) can be stored at room temperature (18-25°C) for 2-3 hours. For short time storage (up to 24 h) samples should be stored at 2-8°C. For long-term storage, freezing samples at -20°C or -80°C is recommended. Various blood collection tubes (e.g., Sarstedt, Greiner) and anticoagulants can be used to collect blood samples.

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).

3.3 Short protocol Invisorb® Spin Blood Mini Kit



Lyse samples

1. Transfer 20 µl Proteinase S into the bottom of a 2.0 ml Safe Lock Tube

2. Mix sample well before taking aliquots for preparation.
   Transfer 200 µl of the sample into the 2.0 ml Safe Lock Tube

3. Add 200 µl Lysis Buffer HLT
   15 sec. pulse-vortexing
   Incubate 10 min at 56°C while continuously shaking

Bind genomic DNA

4. Add 200 µl Binding Solution
   15 sec. pulse-vortexing

5. Briefly centrifuge
   Transfer lysate to RTA Spin Filter and incubate 1 min

6. Centrifuge 2 min at 11.000 x g
   Discard the filtrate
   Transfer the RTA Spin Filter to a new RTA Receiver Tube

Wash to remove residual contaminations

7. Add 600 µl Wash Buffer HLT
   Centrifuge 1 min at 11.000 x g
   Discard the filtrate
   Transfer the RTA Spin Filter to a new RTA Receiver Tube

8. Add 700 µl Wash Buffer
   Centrifuge 1 min at 11.000 x g
   Discard the filtrate
   Transfer the RTA Spin Filter to a new RTA Receiver Tube

9. Add 700 µl Wash Buffer
   Centrifuge 1 min at 11.000 x g
   Discard the filtrate
   Put the RTA Spin Filter back to the RTA Receiver Tube

10. Centrifuge 4 min at maximum speed for ethanol removal

Elute DNA

11. Place the RTA Spin Filter into a 1.5 ml Receiver Tube
    Add 30 - 200 µl of Elution Buffer M (preheated to 56°C)
    Incubate 1 min at room temperature

12. Centrifuge 1 min at 11.000 x g to elute DNA

3.4 Protocol: DNA isolation from 1-200 µl human blood or 1-30 µl buffy coat

1. Pipet 20 µl **Proteinase S** into the bottom of a 2.0 ml Safe Lock Tube.
2. Transfer 1 - 200 µl whole blood or 1 – 30 µl buffy coat to the 2.0 ml Safe Lock Tube, always mix the sample well before taking aliquots for preparation.
   If the sample volume is below 200 µl, adjust with 1 x PBS Buffer or DNase/RNase free water to a final volume of 200 µl.
3. Add 200 µl **Lysis Buffer HLT**, mix thoroughly 15 sec. by pulse-vortexing and incubate for 10 min at 56°C while continuously shaking.
4. Add 200 µl **Binding Solution** to the sample and mix thoroughly 15 sec. by pulse-vortexing**.**
5. Briefly centrifuge to remove drops from the inside of the lid.
   Take a RTA Spin Filter Set. Transfer the mixture into the RTA Spin Filter.
   Close the RTA Spin Filter and incubate for 1 min.
6. Centrifuge for 2 min at 11.000 x g. Discard the filtrate and place the RTA Spin Filter in a new 2.0 ml RTA Receiver Tube.
7. Add 600 µl **Wash Buffer HLT** to the RTA Spin Filter.
   Centrifuge for 1 min at 11.000 x g .
   Discard the filtrate and place the RTA Spin Filter in a new 2.0 ml RTA Receiver Tube.
8. Add 700 µl **Wash Buffer** and centrifuge for 1 min at 11.000 x g
   Discard the filtrate and place the RTA Spin Filter in a new 2.0 ml RTA Receiver Tube.
9. Add 700 µl **Wash Buffer** and centrifuge for 1 min at 11.000 x g.
   Discard the filtrate.
10. Place the RTA Spin Filter **back to** the 2.0 ml RTA Receiver Tube.
    Centrifuge for 4 min at maximum speed to eliminate the ethanol completely.
11. Place the RTA Spin Filter in a 1.5 ml Receiver Tube.
    Add 30-200 µl of the preheated (56°C) **Elution Buffer M**.
    Incubate at room temperature for 1 min.
12. Centrifuge at 11.000 x g for 1 min. Discard the RTA Spin Filter.

Note: The determination of the elution volume depends on the expected yield of genomic DNA. However, pay attention that the minimum volume of Elution Buffer M is 30 µl. Using the minimum volume can reduce the maximum yield. If a large amount of DNA is expected, the volume of Elution Buffer M can be increased.

3.5 Supplemental Protocol (RUO): DNA isolation from bone marrow

Preparation of the starting material:

Fresh material:

- 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 (not provided)
- Scrape cytological material into the Receiver Tube using the edge of a clean slide
- Dissolve the resulting sludge by pipetting up and down.

Sample Lysis

1. Pipet 20 µl **Proteinase S** into the bottom of the 2.0 ml Safe Lock Tube
2. Transfer the starting material into the 2.0 ml Safe Lock Tube. Equilibrate with 1 x PBS Buffer e.g. to 200 µl.
3. Add 200 µl **Lysis Buffer HLT**, mix thoroughly 15 sec. by vortexing and incubate for 3 min at 56°C while continuously shaking.

   Note*: Do not add the **Proteinase S** directly to the **Lysis Buffer HLT***
4. Incubate the reaction tube for 5 min at 56°C while continuously shaking.

   Note*: If you are using a water bath, please vortex the sample 2-5 times during lysis.*

Proceed as described in protocol 1 steps 4 – 12.

4. Appendix

4.1 Troubleshooting

Problem	Possible cause	Recommendation
Low amount of DNA	Insufficient cell lysis	Increase lysis time with **Lysis Buffer HLT** Continuously shaking improves lysis efficiency Reduce amount of starting material to avoid column overload
	Insufficient cell lysis due to decreased **Proteinase S** activity	Ensure, that **Proteinase S** is not added directly to **Lysis Buffer HLT**
	Insufficient lysis due to insufficient mixing with **Lysis Buffer HLT**	Repeat the DNA purification procedure with a new sample. Be sure to mix the sample and **Lysis Buffer HLT** immediately and thoroughly by pipetting up and down 10 times or by pulse-vortexing.
	Usage of ethanol with lower percentage than 96 - 100% or denatured ethanol	Repeat purification with a new sample, use the correct ethanol percentage
	Incomplete elution	Increase incubation time with preheated **Elution Buffer M** to 5-10 min Elute twice with 100 µl **Elution Buffer M** Use higher volume of **Elution Buffer M**
	Low DNA-concentration in the sample	Elute the DNA with a lower volume of **Elution Buffer M**, do not use volumes lower than 30 µl
	Water with wrong pH (acidic) was used for elution	Low pH may reduce DNA yield. Ensure that the pH of the water is at least 7.0 or use **Elution Buffer M** (contains 10 mM Tris-HCL, no EDTA)
	No **Binding Solution** added to the lysate before loading onto the RTA Spin Filter	Repeat the purification procedure with a new sample
Colored residues on the RTA Spin filter after washing	Insufficient cell lysis	See above
	Inefficient washing	Wash again with **Wash Buffer**
	Wash Buffer HLT and **Wash Buffer** incorrectly prepared	Ensure that **Wash Buffer HLT** and **Wash Buffer** were diluted with the correct volume of pure isopropanol or ethanol. Repeat the purification with a new sample.
Degraded or sheared DNA	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

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 deviates 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 Blood Mini Kit	250 preparations	1031100300

Revision history

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

INVITEK
Molecular

Invitek Molecular GmbH
Robert-Rössle-Str. 10
13125 Berlin
Germany

Phone: +49 30 9489 2908
Fax: +49 30 9489 3795
info@invitek-molecular.com

www.invitek-molecular.com

https://www.invitek-molecular.com/resources/manuals.html

2022-05-18 EN-v1-2022