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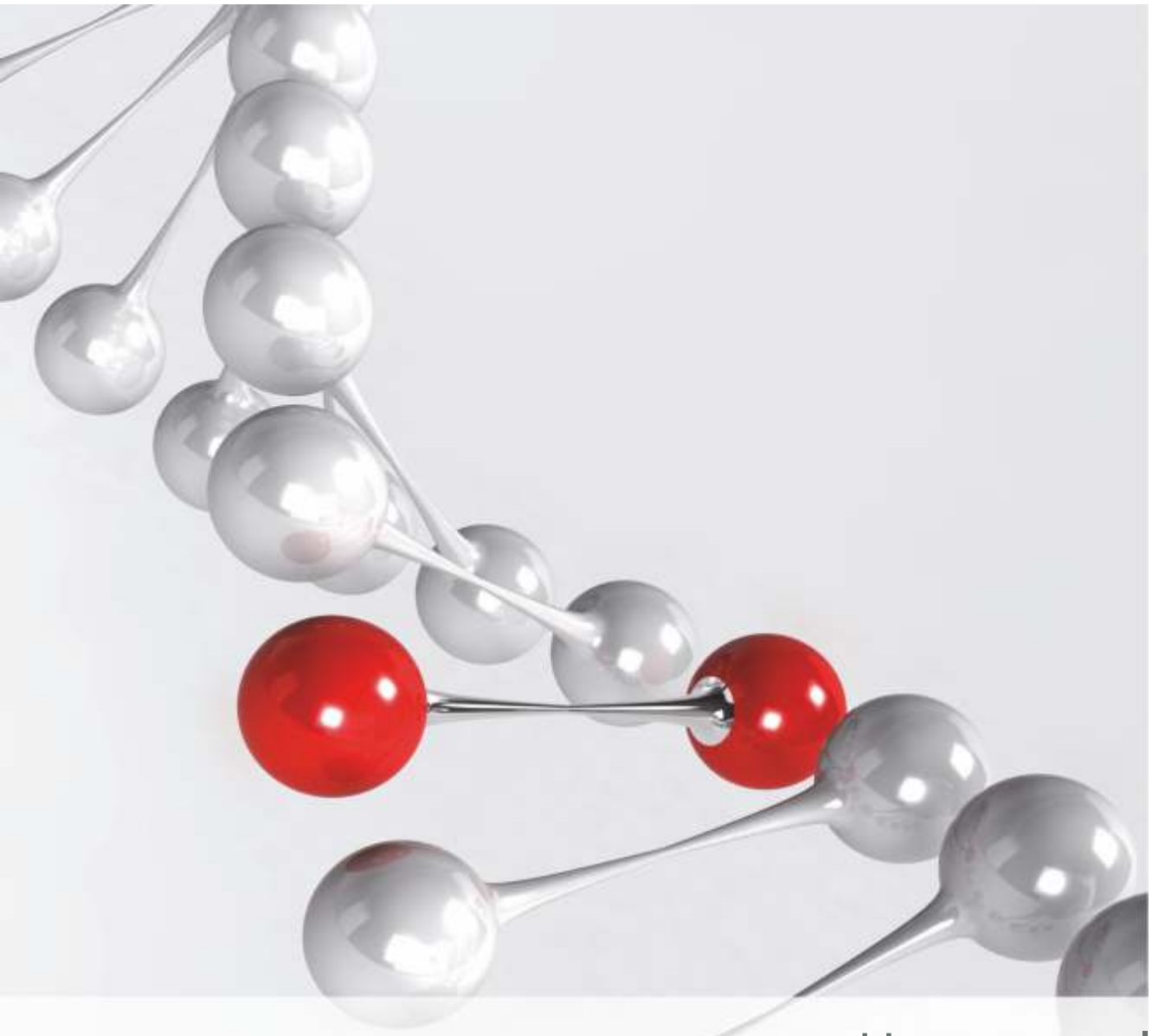

User manual
Invisorb® Spin Blood Mini Kit

For genomic DNA purification from fresh, frozen or old human blood with common anticoagulants (EDTA, Citrate) as well from buffy coat and bone marrow

CE
IVD

REF 1031100x00

STRATEC Molecular GmbH, D-13125 Berlin

Instruction for the Invisorb® Spin Blood Mini Kit

The **Invisorb® Spin Blood Mini Kit** is the ideal tool, using the Invisorb® technology for a fast, efficient and simple manual isolation and purification of genomic DNA from max. 200 µl fresh, or frozen (-20°C or -70°C) human blood with common anticoagulants (EDTA, Citrate) as well from buffy coat (max. 30 µl). The purified DNA can be used for in-vitro diagnostic analysis.

The kit may be used for isolation of genomic DNA from max. 200 µl of non human mammalian blood* or from up to 25 µl non mammalian blood e.g. birds or fishes*.

The kit is neither validated for the isolation of genomic DNA from tissue, serum, plasma, synovial fluid and urine, nor from bacteria, stool sample, fungi, parasites or the purification of total RNA

The application of the kit for isolation and purification of viral DNA has not been evaluated.

*Protocols are only validated for human blood, adjustment of the process may be necessary

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

* Protocols may need adaption, due to the specific properties of the respective starting materials, for further information please contact support (supoportadresse)

Kit contents of the Invisorb® Spin Blood Mini Kit

	5 DNA extractions	50 DNA extractions	250 DNA extractions
Catalogue No.	1031100100	1031100200	1031100300
Lysis Buffer HLT	2 ml	15 ml	60 ml
Binding Solution (fill with 99.7% Isopropanol)	2 x 1 ml (ready to use)	empty bottle (final volume 15 ml)	empty bottle (final volume 80 ml)
Elution Buffer M	2 ml	15 ml	60 ml
Proteinase S	2 ml	2 ml	3 x 2 ml
Wash Buffer HLT	15 ml (ready to use)	30 ml (final Volume 50 ml)	105 ml (final Volume 175 ml)
Wash Buffer	15 ml (ready to use)	2 x 18 ml (final volume 2 x 60 ml)	3 x 45 ml (final volume 3 x 150 ml)
RTA Spin Filter Set	5	50	5 x 50
2.0 ml Safe–Lock-Tubes	5	50	5 x 50
RTA Receiver Tubes	15	3 x 50	15 x 50
1.5 ml Receiver Tubes	5	50	5x 50
Manuals	1	1	1
Initial steps	.	Fill 15 ml 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle Add 20 ml of 99.7% **Isopropanol** to the bottle **Wash Buffer HLT.** Mix thoroughly and always keep the bottle firmly closed Add 42 ml of 96 -100% ethanol to **Wash Buffer**, mix thoroughly and always keep the bottle firmly closed!	Fill 80 ml 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle Add 70 ml of 99.7% **Isopropanol** to the bottle **Wash Buffer HLT.** Mix thoroughly and always keep the bottle firmly closed Add 105 ml of 96 -100% ethanol to **Wash Buffer**, 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 Blood Mini Kit** 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).

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

Wash Buffer HLT and **Binding Solution** charged with isopropanol should be appropriately sealed and stored at room temperature.

Quality control and product warranty

STRATEC Molecular warrants the correct function of the **Invisorb® Spin Blood 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, STRATEC Molecular will check the lot and if STRATEC Molecular investigates a problem in the lot, STRATEC Molecular will replace the Product free of charge.
STRATEC Molecular reserves the right to change, alter, or modify any Product to enhance its performance and design at any time.
In accordance with STRATEC Molecular's EN ISO 9001 and EN ISO 13485 certified Quality Management System the performance of all components of the **Invisorb® Spin Blood 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 Blood Mini Kit** or other STRATEC Molecular products, please do not hesitate to contact us. A copy of STRATEC Molecular's terms and conditions can be obtained upon request or are presented at the STRATEC Molecular webpage.

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 Blood Mini Kit** is the ideal tool for a fast and convenient manual isolation and purification of genomic DNA from max. 200 µl fresh or frozen human blood as well from buffy coat (max. 30 µl). For reproducible and high yields appropriate sample storage is essential. The purified DNA can be used for in-vitro diagnostic analysis only.

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

The protocol for the isolation and all buffers are optimized for a high yield as well as a high purity. All hands on steps are 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 is in compliance with EU Directive 98/79/EC on in vitro medical devices. But it is not for in vitro diagnostic use in countries where the EU Directive 98/79/EC on in vitro medical devices is not recognized.

Product use limitation

The kit is neither validated for the isolation of genomic DNA from tissue, serum, plasma, synovial fluid and urine, nor from bacteria, stool sample, fungi, parasites or the purification of total RNA

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 STRATEC Molecular Product for any particular use. STRATEC Molecular does not provide for validation of performance characteristics of the Product with respect to specific applications.

STRATEC 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.

This product sold by STRATEC Molecular is subject to extensive quality control procedures (according to EN ISO 9001 and EN ISO 13485) and is warranted to perform as described herein. Any problems, incidents or defects shall be reported to STRATEC 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.molecular.stratec.com** for each STRATEC 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.

STRATEC Molecular has not tested the liquid waste generated by the **Invisorb® Spin Blood Mini** 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 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

Wash Buffer HLT (ready to use)

Danger

H225-H315-H336-P403+P233

Wash Buffer (ready to use)

Danger

H225-P403+P233

H225: Highly flammable liquid and vapour.
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.
H336: May cause drowsiness or dizziness.
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
P403+P233: Store in a well-ventilated place. Keep container tightly closed.

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 Blood Mini Kit

Starting material	Yield	Time for preparation	Ratio
1 - 200 µl fresh or frozen human whole blood (EDTA, citrate) 1 – 30 µl buffy coat	up to 10 µg (in average about 6 µg) depends on amount of lymphocytes, sample source, sample transport, sample storage, and age of the sample	approx. 25 min	A_{260} : A_{280} 1.7 – 2.0

The **Invisorb® Spin Blood Mini Kit** provides a very efficient procedure for isolation of high quality DNA directly from fresh, frozen, or old blood samples treated with citrate or EDTA or buffy coat samples.

The kit is designed for simultaneous processing of multiple samples. Prior separation of leukocytes is not necessary.

The whole blood sample is lysed in an optimized lysis buffer and proteins are degraded during the lysis with **Proteinase S**. The DNA binds to filter membrane, followed by washing steps and the final elution.

The purification procedure is rapid and requires neither phenol/ chloroform extraction nor alcohol precipitation, and requires minimal interaction by the user, allowing safe handling of potentially infectious samples. The procedure is designed to avoid sample-to-sample cross-contamination. Due to the high purity, the isolated genomic DNA is ready to use for a broad panel of downstream applications (see below) or can be stored at –20°C for subsequent use.

Downstream Application:
- PCR *)
- Restriction Enzyme Digestion
- SNP Analysis
- HLA typing
- Cloning

To purify genomic DNA in 96 format STRATEC Molecular offers the **Invisorb® Blood Mini HTS 96 Kit** for use in a centrifuge and on common laboratory automated workstations. Furthermore STRATEC Molecular offers the **InviMag® Blood Mini Kits** for DNA isolation using magnetic beads.

To purify genomic DNA from large volumes of blood STRATEC Molecular offers the **Invisorb® Spin Blood Midi Kit** (max. 2 ml) and the **Invisorb® Blood Universal Kit** (1 – 10 ml).

For blood stains STRATEC Molecular offers the **Invisorb® Spin Forensic Kit.**

For further information please contact: Tel.: +49 (0) 30 9489 2901, 2910 in Germany and from foreign countries Tel.: +49 (0) 30 9489 2903/ 2907 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 Blood Mini Kit** procedure comprises following steps:

1. lysis of sample material
2. binding the genomic DNA to the membrane of RTA Spin Filter
3. washing the membrane and elimination of ethanol
4. elution of genomic DNA

This manual contains 3 protocols, according to the different requirements of the starting materials.

Sampling and storage of starting material

Blood and Buffy Coat

Mammalian blood samples (stabilized with EDTA or Citrate) can be stored at room temperature 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 system (e.g. Sarstedt, Greiner) and anticoagulants (except heparin) can be used to collect blood samples for the **Invisorb®** procedure.

Buffy coat is a whole-blood fraction of enriched leukocyte cells. To prepare and extract a buffy coat layer the following procedure is recommended. The use of a whole blood sample (anticoagulants: EDTA, citrate, *not heparin*) with a sedimented cellular fraction from staying overnight at 4°C is recommended. The resulting bright mid-section overlaid by the clear plasma is buffy coat containing concentrated leukocytes that can be easily distinguished from the erythrocytes in the bottom layer. An enrichment factor of 10 is expected from such a procedure. Due to the enriched leukocyte content be aware to avoid overloading the DNA purification procedure.

STRATEC 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 at elevated temperatures. Lysis is performed in the presence of **Lysis Buffer HLT** and **Proteinase S.**
Proteinase S is colored blue; this is for tracking the correct addition of the small volume enzyme.

Binding genomic DNA

By adding **Binding Solution** to the lysate, optimal binding conditions will be adjusted. Each lysate is then applied to an RTA Spin Filter and genomic DNA is adsorbed to the membrane.

Removing residual contaminations

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

Elution
Genomic DNA is eluted from the RTA Spin Filter using 30 - 200 µl **Elution Buffer M**. The eluted DNA is ready for use in different downstream applications. Eluted DNA stored at 4 – 8°C is stable for 2 months, for more than 5 years if stored at -20°C.

Yield and quality of genomic DNA

The amount of purified DNA using the **Invisorb® Spin Blood Mini Kit** procedure depends on the sample type and the number of cells in the sample (depending from the patient's age and health situation, sample source, transport conditions, storage, and age of the sample).

Typically, a 200 µl sample of whole blood cells from a healthy individual will yield 3–10 µg of DNA. (If higher yields are required, use **Invisorb® Blood Universal Kit** with up to 10 ml blood, respectively). Samples with elevated white blood cell (WBC counts, ranging from 3 x 10^6 to 1 x 10^7cells/ml) give a higher yield.

For most whole blood samples, a single elution with 200 µl **Elution Buffer M** is sufficient. For samples with elevated white blood cell approximately 80% of the DNA will elute in the first 200 µl, and up to 20% more in the next 200 µl.

Yield and quality of isolated genomic DNA is suitable for any molecular-diagnostic detection system. The diagnostic tests should be performed according to manufacturers' specifications.

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

- Always change pipet tips between liquid transfers. To avoid cross-contamination, we recommend the use of aerosol-barrier pipet tips.
- All centrifugation steps should be 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 personnel.

Preparing reagents and buffers

1. Adjust the thermomixer to 56°C.
2. Warm up the needed amount of **Elution Buffer M** to 56°C (100 - 200 µl **Elution Buffer M** are needed per sample).
3. Label the needed amount of RTA Spin Filter (lid).
4. Label the needed amount of 1.5 ml Receiver Tubes (per sample: 1 Receiver Tube).
5. Add the needed amount of ethanol to the **Wash Buffer HLT** and **Wash Buffer.**

5 DNA extractions:
Wash Buffer HLT and **Wash Buffer** are ready to use
50 DNA extractions:
Fill 15 ml 99.7% Isopropanol (molecular biologic grade) into the empty bottle Add 20 ml of 99.7% **Isopropanol** to the bottle **Wash Buffer HLT.** Mix thoroughly and always keep the bottle firmly closed Add 42 ml of 96 -100% ethanol to **Wash Buffer**, mix thoroughly and always keep the bottle firmly closed!
250 DNA extractions:
Fill 80 ml 99.7% Isopropanol (molecular biologic grade) into the empty bottle Add 70 ml of 99.7% **Isopropanol** to the bottle **Wash Buffer HLT.** Mix thoroughly and always keep the bottle firmly closed Add 105 ml of 96 -100% ethanol to **Wash Buffer**, 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.molecular.stratec.com under each STRATEC Molecular kit and kit component.

- Microcentrifuge
- Thermomixer (for 56°C)
- Measuring cylinder (250 ml)
- Disposable gloves
- Pipette and pipette tips
- Vortexer
- Reaction tubes (1.5 ml or 2.0 ml)
- dd H_2O
- 96 - 100 % ethanol
- 1 x PBS (optional)
- Isopropanol*

*The **Invisorb® Spin Blood 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

Important indications

1. Process only as much blood samples as the microcentrifuge allows to process.

2. Blood samples and buffers should be thoroughly mixed and should have room temperature

3. The elution can be done by using lower amount of **Elution Buffer M**. This may result in a higher concentration of DNA. But pay attention that minimum volume for elution is 30 µl, but this will reduce the yield. Elution volume between 2 x 30 µl up to 200 µl will realize comparable results.

4. The eluted DNA volume can be lower than the added **Elution Buffer M** volume. **Elution Buffer M** should be preheated to 56 °C.

5. The **Elution Buffer M** doesn't contain EDTA.

6. The yield can be increased if the incubation time with preheated **Elution Buffer M** 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 Spin Filter membrane.

Scheme of the Invisorb® Spin Blood Mini Kit

genomic DNA

Please read protocols prior the start of the preparation carefully

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

Transfer max. 200 µl of the blood into this 2.0 ml Safe Lock Tube

Add 200 µl Lysis Buffer HLT and mix thoroughly 15 sec. by pulse-vortexing

Incubate for 10 min at 56°C while continuously shaking

Add 200 µl Binding Solution and mix thoroughly 15 sec. by pulse-vortexing
Briefly centrifuge the 2.0 ml Safe Lock Tube

Take a RTA Spin Filter Set
Transfer lysate onto RTA Spin Filter and 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 600 µl Wash Buffer HLT
Centrifuge for 1 min at 11.000 x g (11.000 rpm)
Discard the filtrate and the RTA Receiver Tube

Place RTA Spin Filter to a new 2.0 ml RTA Receiver Tube
Add 700 µl Wash Buffer
Centrifuge for 1 min at 11.000 x g (11.000 rpm)
Discard the filtrate and the RTA Receiver Tube
repeat the step, but add the RTA Spin Filter to the same RTA Receiver Tube
then centrifuge for 4 min at maximum speed for ethanol removal

Place the RTA Spin Filter again into the 2.0 ml RTA Receiver Tube. Centrifuge for
4 min at maximum speed to eliminate the ethanol completely

Place the RTA Spin Filter into a 1.5 ml Receiver Tube
Add 100 - 200 µl of Elution Buffer M (preheated to 56°C)
Incubate for 1 min at room temperature
Centrifuge for 1 min at 11.000 x g (11.000 rpm)
Discard RTA Spin Filter
Close the 1.5 ml Receiver Tube and store the DNA sample at 4 °C

Protocol : DNA Isolation from 1 - 200 µl human whole blood or 1 – 30 µl buffy coat

Please read the instructions carefully and conduct the prepared procedure.

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

1. Pipet 20 µl **Proteinase S** into the bottom of the 2.0 ml Safe Lock Tube
2. Transfer 1 - 200 µl whole blood or 1 – 30 µl buffy coat into this 2.0 ml Safe Lock Tube. If sample volume is lower than 200 µl, equilibrate with 1 x PBS Buffer or distilled water to 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.

Note*: Do not add the Proteinase S directly to the Lysis Buffer HLT*

4. Add 200 µl **Binding Solution** to the sample and mix thoroughly 15 sec. by pulse- vortexing**.**
5. Briefly centrifuge the 2.0 ml Safe Lock Tube 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 (11.000 rpm). 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. Close the RTA Spin Filter. Centrifuge for 1 min at 11.000 x g (11.000 rpm). 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 (11.000 rpm). 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 (11.000 rpm). Discard the filtrate.
10. Place the RTA Spin Filter again into 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 200 µl of the preheated (56°C) **Elution Buffer M**. Incubate at room temperature for 1 min.
12. Centrifuge at 11.000 x g (11.000 rpm) for 1 min. Discard the RTA Spin Filter.

Note: *The DNA can also be eluted with a lower volume of Elution Buffer M (depends on the expected yield of genomic DNA). But pay attention that the minimum volume for the elution is **30 µl and that this volume can reduce the maximum yield**. If quite large amount of DNA is expected, the volume of Elution Buffer M can be increased.*

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

This protocol also may be used for mammalian blood, changes of the amounts of sample material (sample volume < 200 µl) and lysis time may be necessary /please contact technical support, see page 3)

Proposal 1: DNA Isolation from non-mammalian blood

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.

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

1. Pipet 20 µl **Proteinase S** into the bottom of the 2.0 ml Safe Lock Tube
2. Transfer 1 - 25 µl whole blood into this 2.0 ml Safe Lock Tube.
   If sample volume is lower than 200 µl, equilibrate with 1 x PBS Buffer or distilled water to 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.

Note*: Do not add the Proteinase S directly to the Lysis Buffer HLT*

4. Add 200 µl **Binding Solution** to the sample and mix thoroughly 15 sec. by pulse- vortexing**.**
5. Briefly centrifuge the 2.0 ml Safe Lock Tube 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 (11.000 rpm). 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.
   Close the RTA Spin Filter.
   Centrifuge for 1 min at 11.000 x g (11.000 rpm).
   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 (11.000 rpm).
   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 (11.000 rpm).
   Discard the filtrate.
10. Place the RTA Spin Filter again into 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 200 µl of the preheated (56°C) **Elution Buffer M**. Incubate at room temperature for 1 min.
12. Centrifuge at 11.000 x g (11.000 rpm) for 1 min. Discard the RTA Spin Filter.

Note: *The DNA can also be eluted with a lower volume of Elution Buffer M (depends on the expected yield of genomic DNA). But pay attention that the minimum volume for the elution is **30 µl and that this volume can reduce the maximum yield**. If quite large amount of DNA is expected, the volume of Elution Buffer M can be increased.*

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

Proposal 2: DNA isolation from bone marrow

For the isolation and purification of DNA from small amounts of various human and mammalian samples

Please read the instructions carefully and conduct the prepared procedure!

Important *Transfer the needed amount of Elution Buffer M 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:

- 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.

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

4. Incubate the reaction tube for 5 min at 56°C while continuously shaking on a thermomixer.

Note: *Do not add the Proteinase S directly to the Lysis Buffer HLT*

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

Proceed as described in protocol 1 steps 4 – 12.

Troubleshooting

Problem	Cause	Comments and suggestions
low amount of DNA	insufficient cell lysis	increase lysis time with **Lysis Buffer HLT** reduce amount of starting material continuously shaking improves lysis efficiency
	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 5 times or by pulse-vortexing
	inefficient binding of DNA to the membrane	overloading RTA Spin Filter reduces yield
	low percentage alcohol used instead of 96 - 100%	repeat purification procedure with a new sample with the correct percentage
	incomplete elution	increase incubation time with preheated **Elution Buffer M** t 5 - 10 min elute twice with each 100 µl **Elution Buffer M** use higher volume of **Elution Buffer** M.
	low DNA-concentration in the sample	elute the DNA with lower volume of **Elution Buffer M**
	pH of water incorrect (acidic)	low pH may reduce DNA yield. Ensure that the pH of the water is at least 7.0 or use **Elution Buffer M** (contains only 10 mM Tris – HCL, no EDTA)
colored residues remain on the RTA Spin filter after washing	insufficient cell lysis	see above
	no **Binding Solution** added to the lysate before loading onto the RTA Spin Filter	repeat the purification procedure with a new sample
	inefficient washing	wash again with **Wash Buffer**
	Wash Buffer HLT and **Wash Buffer** prepared incorrectl	ensure that **Wash Buffer HLT** and **Wash Buffer** concentrates 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 harvested and stored as described or page 7

Problem	Cause	Comments and suggestions
clogged Spin Filter	incorrect storage of starting material	blood stored longer time at RT may form clumps of proteins etc. Prevent a transfer of this clumps into the sample cryoprecipitates have formed in blood due to related freezing and thawing, do not use blood that has been frozen and thawed more than once, prevent a transfer of cryoprecipitates into the sample
	insufficient lysis	increase lysis time with **Lysis Buffer HLT**
	too much starting material	concentration of leukocytes in samples was greater than 5×10^6/ 200 µl
problems with subsequent applications (e.g. in PCR)	ethanol in the eluted DNA	verify if the recommended centrifugation time was reached increase centrifugation time for the elimination of ethanol if necessary
	salt in the eluate	**Wash Buffer** should be stored used at RT verify **Wash Buffer** on the precipitation of salt. If there are precipitations dissolve this by careful warming up to 30°C
	reduced sensitivity of amplification reaction	adjust the volume of eluate added as template in the amplification reaction
A_{260}/A_{280} ratio for purified DNA is high	high level of residual RNA	in future DNA preparations prepare an RNase digestion step add 10 µl **RNase A** (10 mg/ ml) to the eluted DNA, vortex shortly and incubate 10 min at RT
A_{260}/A_{280} ratio for purified DNA is low	insufficient lysis due to insufficient mixing with **Lysis Buffer HLT**	see above under “low amount of DNA”

Appendix

General notes on handling DNA

Starting material

This kit is designed for extraction of DNA from 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 lyses conditions of blood differ 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 and long-template PCR.

Storage of DNA

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

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

Drying, dissolving and pipetting DNA

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

DNA Yield

The amount of purified DNA from the whole blood depends on the leucocytes content, 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 Blood Mini Kit	5 preparations	1031100100
Invisorb® Spin Blood Mini Kit	50 preparations	1031100200
Invisorb® Spin Blood Mini Kit	250 preparations	1031100300
Lysis Buffer HLT	15 ml	1031101800
Wash Buffer HLT (add 20 ml Isopropanol)	30 ml	1031103800
Wash Buffer (add 42 Ethanol ml)	18 ml	1031103200
Elution Buffer M	15 ml	1031104000
Proteinase S	2 ml	3020420100
Invisorb® Spin Blood Midi Kit	50 preparations	1031110300
Invisorb® Blood Universal Kit	500 ml	1031150200
Invisorb® Blood Universal Kit	1000 ml	1031150300
Invisorb® Blood Mini 96 HTS/ C	4 x 96 preparations	7031300300
Invisorb® Blood Mini 96 HTS/ C	24 x 96 preparations	7031300400
InviMag® Blood DNA Mini Kit/ KFml	15 preparations	2431110100
InviMag® Blood DNA Mini Kit/ KFml	75 preparations	2431110200
InviMag® Blood DNA Mini Kit/ KF96	1 x 96 preparations	7431300100
InviMag® Blood DNA Mini Kit/ KF96	5 x 96 preparations	7431300200

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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