

User manual Invisorb[®] Blood Universal Kit

For purification of genomic DNA from 1 – 10 ml of whole mammalian blood (with the common anticoagulants: EDTA, citrate)



REF 1031150x00



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Instruction for the Invisorb® Blood Universal Kit

The **Invisorb® Blood Universal Kit** provides a very fast, efficient, and careful procedure for the isolation of high quality DNA from 1 – 10 ml of whole blood or from buffy coat. The procedure can be used for native blood, as well as for blood treated with common anticoagulants (EDTA, citrate). The concentration of the final DNA can be adapted to the individual needs of the user (for instance very highly concentrated DNA for HLA typing). The purified DNA can be used for *in vitro* diagnostic analysis.

The kit is neither validated for the isolation of genomic DNA from cultured or isolated cells, from tissue, swabs, dried blood stains, or cell free body fluids, like cerebrospinal fluid, synovial fluid and urine, stool sample, nor from bacteria, fungi, parasites or the purification of total RNA.

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



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.

Trademarks: Invisorb®, Registered marks, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

The Invisorb® technology is covered by patents and patent applications: US 6,110,363, US 6,043,354, US 6,037,465, EP 0880535, WO 9728171, WO 9534569, EP 0765335, DE 19506887, DE 10041825.2, WO 0034463.

Invisorb® is a registered trademark of STRATEC Biomedical AG.

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

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Kit contents of the Invisorb® Blood Universal Kit

Store dissolved Proteinase K at –20°C!

Store all other kit components at room temperature (RT)!

	for preparation from 50 x 1 ml up to 5 x 10 ml	for preparation from 500 x 1 ml up to 50 x 10 ml	for preparation from 1000 x 1 ml up to 100 x 10 ml
Catalogue No.	1031150100	1031150200	1031150300
Buffer EL	250 ml (ready to use)	3 x 30 ml (final volume 1000 ml)	6 x 30 ml (final volume 1000 ml)
Lysis Buffer HL	30 ml	2 x 140 ml	2 x 270 ml
Proteinase K	for 300 µl working solution	for 3 x 1 ml working solution	for 5 x 1.1 ml working solution
Precipitation Solution (fill with 99.7% Isopropanol)	empty bottle (final volume 30 ml)	2 empty bottles (final volume 2 x 140 ml)	2 empty bottles (final volume 2 x 270 ml)
Buffer U	15 ml	2 x 60 ml	250 ml
Manuals	1	1	1
Initial steps	<p>Fill 30 ml 99.7% Isopropanol (molecular biologic grade) into the empty bottle</p> <p>Add 300 µl ddH₂O to Proteinase K, mix thoroughly and store at -20°C!</p>	<p>Fill 140 ml 99.7% Isopropanol (molecular biologic grade) into each empty bottle</p> <p>Add 1 ml ddH₂O to Proteinase K, mix thoroughly and store at -20°C!</p> <p>Mix thoroughly 970 ml of ddH₂O with the 30 ml Buffer EL concentrate and keep the bottle always firmly closed!</p>	<p>Fill 270 ml 99.7% Isopropanol (molecular biologic grade) into each empty bottle</p> <p>Add 1.1 ml ddH₂O to Proteinase K, mix thoroughly and store at -20°C!</p> <p>Mix thoroughly 970 ml of ddH₂O with the 30 ml Buffer EL concentrate and keep the bottle always firmly closed!</p>

Symbols

	Manufacturer
	Lot number
	Catalogue number
	Date of manufacture
	Expiry date
	Consult operating instructions
	Temperature limitation
	Do not reuse

Storage

All buffers and kit components of the **Invisorb® Blood Universal Kit**, except dissolved **Proteinase K** should be stored at room temperature (RT) and are stable for 12 months under these conditions. **Room temperature (RT) is defined as range from 15 - 30°C.**

Dissolved **Proteinase K** stored at –20°C is stable for 12 months, but repeated freezing and thawing should be avoided. Aliquotation and storage at – 20°C is recommended.

If there are any precipitates within the provided solutions dissolve these precipitates by carefully warming up to room temperature (up to 30°C).

Quality control and product warranty

STRATEC Molecular warrants the correct function of the **Invisorb® Blood Universal 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 ISO 9001-2000 and ISO EN 13485 certified Quality Management System the performance of all components of the **Invisorb® Blood Universal 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® Blood Universal 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
or contact your local distributor.**

from abroad +49-(0)30-9489-2907

Intended use

The **Invisorb® Blood Universal Kit** provides a very fast, efficient, and careful procedure for the isolation of high quality DNA from 1 – 10 ml of whole blood or from corresponding amount of buffy coat. The procedure can be used for native blood, as well as for blood treated with EDTA or citrate, *but not with heparin*, from common blood collection systems. The concentration of the final DNA can be adapted to the individual needs of the user (for instance very highly concentrated DNA for HLA typing). The high purity of the genomic DNA guarantees storage stability at 4°C or at -20°C for several months without degradation. Hence it enables the possibility to ship the DNA without any problems. For reproducible and high yields appropriate sample storage is essential.

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.

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 differs depending on 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.

THE PRODUCT IS INTENDED FOR USE BY PROFESSIONAL USERS 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 cultured or isolated cells, from tissue, stool sample, swabs, dried blood stains, or cell free body fluids, like cerebrospinal fluid, synovial fluid, and urine nor from bacteria, fungi, parasites, or the purification of RNA.

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

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 conditioned upon the complete diagnostic system of the laboratory the laboratory has been validated pursuant to CLIA' 88 regulations in the U.S. or equivalents in other countries.

All Products sold by STRATEC Molecular are subject to extensive quality control procedures (according to ISO 9001-2000 and ISO EN 13485) and are 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.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® Blood Universal 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® Blood Universal Kit** to which they apply, are listed below as follows:

Proteinase K



danger

H315-319-334-335 P280-305-351-338-310-405

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.
P310:	Immediately call a POISON CENTER or doctor/physician.
P405:	Store locked up.

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® Blood Universal Kit

starting material	yield	time	ratio
up to 10 ml fresh, frozen or old whole blood (EDTA, citrate), corresponding amount of buffy coat	up to 400 µg, depends on amount of lymphocytes, sample source, -transport, -storage and sample age	approx. 45 min	$A_{260} : A_{280}$ 1.7 – 1.9

The **Invisorb® Blood Universal Kit** provides an alternative approach for the isolation of DNA from 1.0 - 10 ml mammalian whole blood etc. Traditionally, preparing such DNA from blood requires removal of haemoglobin by labor intensive methods such as density gradients and removal of proteins and lipids with hazardous solvents, like phenol/chloroform (Sambrook et al. 1989). The procedure of the **Invisorb® Blood Universal Kit** is faster to perform than homemade or other commercially available methods. The simple purification procedure is performed without spin filter in a single tube, which reduces costs and simplifies handling.

The kit uses a special lysis reagent for selective lysis of erythrocytes. After all erythrocyte components are removed, leukocyte DNA can be isolated free of interfering hemoglobin. The remaining leukocytes are lysed with optimized buffer and proteins are removed by protein digestion. DNA is precipitated selectively by addition of Precipitation Solution, recovered by centrifugation, washed, and dried. DNA is resuspended in elution buffer and is ready for direct use in downstream assays or for storage at -20°C. The concentration of the resulting DNA can be adapted to the needs of the user. The isolated DNA is highly chromosomal and well suitable for archiving.

Downstream Applications

- PCR *), expand long template PCR
- HLA typing
- Restriction Enzyme Digestion
- Cloning
- Southern Blot

The procedure requires no phenol/ chloroform extraction 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.

To purify genomic DNA from other volumes of whole blood STRATEC Molecular offers the **Invisorb® Spin Blood Mini Kit** (max. 200µl), the **Invisorb® Spin Blood Midi Kit** (max. 2 ml), the **Invisorb® Spin Blood Maxi Kit** (max. 10 ml) and the **Invisorb® Blood Giga Kit** (0.2 – 20 ml).

For minimal amount of starting material the **Invisorb® Spin Micro DNA Kit** (max. 50 µl) is available. For blood stains STRATEC Molecular offers the **Invisorb® Forensic Kit I**.

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

For technical support or further information please contact: +49 (0)30-9489-2907/ 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® Blood Universal Kit** procedure comprises following steps:

1. Removal of erythrocytes
2. Lysis of Lymphocytes
3. Precipitation of DNA, leaving behind contaminants in the supernatant
4. Dissolution of genomic DNA

Sampling and storage of starting material

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 – 4 °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. 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.

Lysis

Samples are lysed at elevated temperature. Lysis is performed in the presence of **Lysis Buffer HL** and **Proteinase K**.

Precipitation of DNA

By adding **Precipitation Solution** to the lysate, optimal precipitation conditions will be adjusted. DNA is separated by centrifugation, contaminants remain in the supernatant.

Suspending DNA

Genomic DNA is dissolved using 400 - 2000 µl **Buffer U**. The dissolved eluted DNA is ready for use in different downstream applications.

Eluted DNA stored at 4 – 8°C is stable for a minimum of 2 months or is stable for more than 5 years stored at -20°C.

Yield and quality of genomic DNA

The amount of purified DNA in the **Invisorb® Blood Universal Kit** procedure from whole blood, depends on the leucocytes content, sample source, transport, storage, and age of the sample. 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 species. Also remember that non mammalian blood contains erythrocytes with nuclei. So for special applications adaptation of starting volumes and lysis time may be recommended.

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

Important notes

Important points before starting a protocol

Immediately upon receipt of the Product, inspect the Product and its components as well as the package for any apparent damages, correct quantities and quality. If there are any unconformities you have to notify 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 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, the use of aerosol-barrier pipet tips is recommended.
- 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 personnel.

Preparing reagents and buffers

1. adjusting the thermomixer to 60°C.
2. add the needed μl ddH₂O to **Proteinase K** and **Buffer EL** (see below). Vortex for 5 sec

for DNA extraction from 50 ml blood
Fill 30 ml 99.7% Isopropanol (molecular biologic grade) into the empty bottle Add 300 μl ddH ₂ O to the tube with Proteinase K Mix thoroughly (vortex 5 sec) and store at -20°C Buffer EL is ready to use!
for DNA extraction from 500 ml blood:
Fill 140 ml 99.7% Isopropanol (molecular biologic grade) into each empty bottle Add 1 ml ddH ₂ O to the tube with Proteinase K Mix thoroughly (vortex 5 sec) and store at -20°C Mix thoroughly 970 ml of ddH ₂ O with 30 ml Buffer EL concentrate and always keep the bottle firmly closed!
for DNA extraction from 1000 ml blood:
Fill 270 ml Isopropanol 99.7% (molecular biologic grade) into each empty bottle Add 1.1 ml ddH ₂ O to the tube with Proteinase K . Mix thoroughly (vortex 5 sec) and store at -20°C! Mix thoroughly 970 ml of ddH ₂ O with 30ml Buffer EL concentrate 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.stratec.com under each STRATEC Molecular kit and kit component.

- Centrifuge
- Thermomixer (for 60°C)
- Ice
- Measuring cylinder (250 ml)
- Disposable gloves
- Pipette and pipette tips
- Vortexer
- dd H₂O
- 70 % ethanol
- Isopropanol *

*The **Invisorb® Blood Universal Kit** is validated with 2-Propanol; Rotipuran >99.7%, p.a., ACS, ISO (Order no. 6752) from Carl Roth

* Possible suppliers for Isopropanol:

Fa. Carl Roth

2-Propanol

Rotipuran >99.7%, p.a., ACS, ISO

Order no. 6752

Fa. Applichem

2-Propanol für die Molekularbiologie

Order no. A3928

Fa. Sigma

2-Propanol

Order no. 59304-1L-F


Important indications

1. The DNA can be concentrated by using lower amounts of **Buffer U**.
Be sure that you dissolve the pellet completely. We recommend the following buffers to dilute the DNA:
 - a) 10 mM Tris HCl/ 0.1mM EDTA; pH 8.5-9.0 or
 - b) 10 mM Tris HCl; pH 8.5-9.0
2. Process only as much blood samples as the centrifuge allows to process
3. It is recommended to mix the isolated DNA after long storage (longer than 24 h) before use in any application.
4. Blood sample and Buffers should be thoroughly mixed and should have room temperature (18 – 25 °C).
5. The Buffer EL has to be cooled down to 4°C (see protocol 2) before use!

Attention ! *If you decant Buffer EI from pelleted lymphocytes, be sure that you don't detach the pellet, depending on the blood* it can be not very well attached.*

*) The composition of plasma and lymphocytes in blood differs depending on age and health status of the patient.

Scheme of the Invisorb® Blood Universal Kit

 <p data-bbox="199 1944 395 1977">genomic DNA</p>	<p data-bbox="557 304 1302 367">Please read protocols prior the start of the preparation carefully</p> <hr data-bbox="557 387 1366 392"/> <p data-bbox="557 439 1334 568">Add needed amount of cold Buffer EL (4 °C) to 15 or 50 ml Reaction Tube Add whole blood sample Mix thoroughly and incubate on ice for 10 min</p> <p data-bbox="557 640 1126 674">Centrifuge at 1000 x g (3.000 rpm) for 5 min</p> <p data-bbox="557 741 1198 804">Remove the supernatant carefully (see page 10) Wash again with Buffer EL (vortex shortly)</p> <p data-bbox="557 875 1406 972">Centrifuge again at 1.000 x g (3.000 rpm) for 5 min Remove the supernatant very carefully, invert the tube and dab it on a paper tissue to remove the residual fluid</p> <p data-bbox="557 1010 1378 1106">Add the needed amount of Lysis Buffer HL and Proteinase K depending on the starting volume of whole blood sample to the cell pellet</p> <p data-bbox="557 1144 1031 1178">Incubate the tube at 60°C for 10 min</p> <p data-bbox="557 1279 1406 1543">Prefill the described amount of Precipitation Solution into 15 ml tubes and add the lysate to the prefilled tubes Mix the tube vigorously by inverting Centrifuge at 2.000 x g (4.500 rpm) for 5 min Remove the supernatant, invert the tube and dab it on paper tissue Add needed amount of 70 % Ethanol to the DNA pellet Vortex shortly</p> <p data-bbox="557 1581 1347 1677">Centrifuge at 2.000 x g (4.500 rpm) for 5 min Remove the supernatant, invert the tube and dab it on paper tissue</p> <p data-bbox="557 1715 1193 1845">Remove ethanol by pipetting or drying Add the needed amount of Buffer U to the pellet Vortex shortly Incubate at 60°C for at least 1 h</p>
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Protocol: Isolation of genomic DNA from whole blood (up to 10 ml) or from buffy coat (corresponding amount)

Please read the instructions carefully and conduct the prepared procedure.

Note: *If buffy coat should be used – start directly with the cell lysis step*

Lysis of Erythrocytes

1. Prefill the described amount of **Buffer EL (4°C)** into 15 ml or 50 ml Reaction Tubes and add the whole blood sample to the **Buffer EL** (see table).

Volume of whole blood (ml)	1	2	3	4	5	6	7	8	9	10
Buffer EL (ml)	2.5	5.0	7.5	10.0	12.5	15.0	17.5	20.0	22.5	25.0
Reaction Tube (ml)	15	15	15	50	50	50	50	50	50	50

2. Mix the sample with the **Buffer EL** very well and place the tube on ice for 10 min.

Note: *If buffy coat is used - for the calculations of volumes in the tables, take the volume of blood from which you made the buffy coat. If you have really clean buffy coat you may start with step 3 from this protocol. If you are not sure that erythrocytes are removed quantitatively, it is recommended to do a treatment with buffer EL starting with step two.*

Collecting of nucleated blood cells

3. Centrifuge at 1.000 x g (3.000 rpm) for 5 min. Remove the supernatant carefully and wash again with the same volume **Buffer EL** as before (vortex shortly).
4. Centrifuge again at 1.000 x g (3.000 rpm) for 5 min. Remove the supernatant very carefully, invert the tube and dab it on a paper tissue to remove the residual fluid (the lymphocytes should be visible at the ground of the tube).

Be careful, don't decant the cell pellet.

Cell Lysis

5. Add the described amount of **Lysis Buffer HL** and **Proteinase K** depending on the starting volume of whole blood sample to the cell pellet (see table).

Volume of whole blood (ml)	1	2	3	4	5	6	7	8	9	10
Lysis Buffer HL (ml)	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0
Proteinase K (µl)	5	10	15	20	25	30	35	40	45	50

Important Note: *When processing multiple blood sample, vortex each tube immediately after addition of **Lysis Buffer HL** and **Proteinase K**. Do not wait until the **Lysis Buffer HL** and **Proteinase K** had been added to all samples!*

6. Incubate the tube at 60°C for 10 min in a water bath or heating block etc. Agitation improves lysis efficiency. If you don't use a thermomixer (small sample volume) it is recommended to mix the sample three time during incubation.

DNA Precipitation

7. Prefill the described amount of **Precipitation Solution** into 15 ml tubes and add the lysate to the prefilled tubes (see table).

Volume of whole blood (ml)	1	2	3	4	5	6	7	8	9	10
Precipitation Solution (ml)	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0

8. Mix the tube vigorously by inverting until the DNA flakes becomes visible.
9. Centrifuge at 2.000 x g (4.500 rpm) for 5 min. Remove the supernatant very carefully, invert the tube and dab it on paper tissue to remove the residual fluid.
Be carefully. Don't decant the pellet.

If the DNA pellets are very loose, centrifugation can be prolonged or the centrifugation speed can be increased.

Washing of the DNA Pellet

10. Add the described amount of 70 % Ethanol to the DNA pellet (see table)

Volume of whole blood (ml)	1	2	3	4	5	6	7	8	9	10
Ethanol 70 % (ml)	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0

11. Vortex shortly.
12. Centrifuge at 2.000 x g (4.500 rpm) for 5 min. Remove the supernatant very carefully, invert the tube and dab it on paper tissue to remove the residual fluid.
Be carefully. Don't decant the pellet.

If the DNA pellets are very loose, centrifugation can be prolonged or the centrifugation speed can be increased.

Drying of the DNA Pellet

13. **From small amounts of blood** (up to 2 ml): Dry the DNA pellet on the air until all traces of ethanol have evaporated. The time for drying should be short as possible (about 5 min).

From bigger amounts of blood (> than 2 ml): Spin down again residual ethanol and remove completely the supernatant with a small-volume pipette.

Attention please ! *Over dried DNA is very difficult to dissolve! The DNA isolated with this kit is of high quality and in big amounts of high complexity, so the dissolving process takes some time.*

Dissolving of the DNA

14. Add the described amount of **Buffer U** to the pellet (see table).

Volume of whole blood (ml)	1	2	3	4	5	6	7	8	9	10
Buffer U (ml)	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8	2.0

15. Vortex shortly (max. 3-5 sec). Dissolve the DNA completely by incubation at 50°C for at least 1 h. The DNA can be also dissolved at room temperature overnight.
Continuously shaking or inverting of the tube from time to time (min. 5 x) increases the dissolving efficiency.
Optional transfer the DNA into a 1.5 ml or 2.0 ml Reaction Tube.

Troubleshooting

Problem	Cause	Comments and suggestions
low amount of DNA	insufficient lysis	increase lysis time with Buffer EL make sure that the Buffer EL is cooled down to 4°C increase lysis time with Lysis Buffer HL continuously shaking improves lysis efficiency.
	incompletely dissolved DNA	increase incubation time with Buffer U
	low DNA-concentration in the sample	dilute the DNA with lower volume of Buffer U
diluted DNA tints yellow	insufficient lysis of erythrocytes	increase lysis time with Buffer EL make sure that the Buffer EL is cooled down to 4°C
	inefficient washing	wash the lymphocyte pellet again with Buffer EL wash again with 70 % ethanol
degraded or sheared DNA	incorrect storage of starting material	ensure the sample is harvested and stored as described avoid repeated thawing and freezing of the material
	old material	old material often contains degraded DNA
problems with subsequent applications (e.g. in PCR)	ethanol in the eluated DNA	spin down the DNA and remove all traces of ethanol by pipetting increase the time for the elimination of ethanol
	salt in the eluat	Check correct calculation of volumes

Appendix

General notes on handling DNA

1) 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 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 lysis time may be recommended.

The procedure can be used for native blood, as well as for blood treated with EDTA or citrate, *but not with heparin*, from common blood collection systems.

2) 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.

3) 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.

4) 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.

5) DNA yield

The amount of purified DNA from the whole blood depends on the leucocytes content, sample source (human, animal, age of the individuals, health situation), transport, storage, and age of the sample. 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® Blood Universal Kit	50 ml preparation	1031150100
Invisorb® Blood Universal Kit	500 ml preparation	1031150200
Invisorb® Blood Universal Kit	1000 ml preparation	1031150300
Invisorb® Spin Blood Mini Kit	50 preparations	1031100200
Invisorb® Spin Blood Mini Kit	250 preparations	1031100300
Invisorb® Spin Blood Maxi Kit	50 preparations	1031120300
Invisorb® Blood Mini HTS 96 Kit/ C	4 x 96 preparations	7031300300
Invisorb® Blood Mini HTS 96 Kit/ C	24 x 96 preparations	7031300400
Invisorb® Blood Mini HTS 96 Kit/ R	4 x 96 preparations	7131300300
Invisorb® Blood Mini HTS 96 Kit/ R	24 x 96 preparations	7131300400
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:

Fa. Carl Roth

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

Fa. Applichem

2-Propanol für die Molekularbiologie
Order no. A3928

Fa. Sigma

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



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