

IBIAN[®] DNA Blood Mini kit



IBIAN TECHNOLOGIES, S.L.

Pol. Ind. Centrovía

C/ San Francisco n. 1

Centro de Negocios, Of. nº 4, 2ª Planta

50196 La Muela - Zaragoza

<http://www.IBIANtech.com>

info@ibiantech.com

IBIAN[®] DNA Blood Mini Kit

For isolation and purification of genomic DNA from:

1-200 µl whole mammalian blood

1-200 µl cerebrospinal fluid

1-30 µl buffy coat

1-25 µl non mammalian blood

1-20 µl bone marrow

Instruction for the IBIAN[®] DNA Blood Mini Kit

The **IBIAN[®] DNA Blood Mini Kit** is the ideal tool, for a fast, efficient and simple manual isolation and purification of genomic DNA from max. 200 µl fresh, frozen or old human blood with common anticoagulants (EDTA, Citrate) as well from buffy coat cerebrospinal fluid and bone marrow (max. 30 µl). The purified DNA can be used for in-vitro diagnostic analysis.

The kit is also useful 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 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.

Contents

Kit contents of the IBIAN[®] DNA Blood Mini Kit	2
Symbols 3	
Storage 3	
Quality Control	3
Intended Use	4
Product Use Limitation	4
Safety Information	4
Product Characteristic of the IBIAN[®] DNA Blood Mini Kit	5
Principle and procedure	6
Sampling and storage of starting material	6
Procedure 6	
Yield and quality of genomic DNA	7
Important points before starting a protocol	7
Preparing reagents and buffers	7
Reagents and equipment to be supplied by user	8
Important indications	8
Scheme 9	

Protocol 1: DNA Isolation from 1 - 200 µl human and mammalian whole blood or 1 – 30 µl buffy coat.....	10
Protocol 2: DNA Isolation from non mammalian blood sample material.....	10
Protocol 3: DNA isolation from CFS and bone marrow.....	11
Troubleshooting.....	13
Appendix	13

Kit contents of the IBIAN® DNA Blood Mini Kit

Store the kit components at room temperature (RT) !

Attention: lyophilized Proteinase K should be stored at 2 – 8 °C, diluted Proteinase K at - 20° C !

	3 DNA extractions	10 DNA extractions	50 DNA extractions	250 DNA extractions
Catalog No.	001BLOOD-003	009BLOOD-010	002BLOOD-050	003BLOOD-250
IBIAN Lysis Buffer HL	1 x 2 ml	2 x 2 ml	1 x 15 ml	1 x 60 ml
IBIAN Binding Buffer HL	1 x 2 ml	2 x 2 ml	1 x 15 ml	1 x 60 ml
IBIAN Elution Buffer D	1 x 2 ml	2 x 2 ml	1 x 15 ml	1 x 60 ml
Proteinase K	for 1 x 250 µl * working solution	for 1 x 250 µl * working solution	for 1 x 1,1 ml * working solution	for 5 x 1,1 ml * working solution
IBIAN Wash Buffer I	1 x 15 ml (ready to use)	1 x 15 ml (ready to use)	1 x 30 ml ** (final volume 60 ml)	1 x 80 ml ** (final volume 160 ml)
IBIAN Wash Buffer II	1 x 15 ml (ready to use)	2 x 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	1 x 3	1 x 10	1 x 50	5 x 50
RTA Receiver Tubes	1 x 9	1 x 30	3 x 50	15 x 50
1.5 ml Receiver Tubes	1 x 6	1 x 20	2 x 50	10 x 50
Manuals	1	1	1	1
Initial steps	* Add 250 µl dd H ₂ O to Proteinase K , mix thoroughly and store at -20°C!	* Add 250 µl dd H ₂ O to Proteinase K , mix thoroughly and store at -20°C!	*Add 1,1 ml dd H ₂ O to Proteinase K , mix thoroughly and store at -20°C! ** Add 30 ml of 96-100% ethanol to IBIAN Wash Buffer I , mix thoroughly and always keep the bottle firmly closed! *** Add 42 ml of 96 - 100% ethanol to IBIAN Wash Buffer II , mix thoroughly and always keep the bottle firmly closed!	*Add 1,1 ml dd H ₂ O to Proteinase K , mix thoroughly and store at -20°C! **Add 80 ml of 96-100% ethanol to IBIAN Wash Buffer I , mix thoroughly and always keep the bottle firmly closed! ***Add 105 ml of 96 - 100% ethanol to IBIAN Wash Buffer II , mix thoroughly and always keep the bottle firmly closed!

Symbols



lot number



catalog number



date of manufacture



expiry date



consult operating instructions



temperature limitation



do not reuse

Storage

All buffers and kit components of the **IBIAN DNA Mini Kit**, except **Proteinase K** should be stored at room temperature (RT) and are stable for at least 12 months under these conditions. The lyophilized **Proteinase K** can be stored at 2-8 °C. Dissolved **Proteinase K** stored at -20°C for at least 12 months, but repeated freezing and thawing should be avoided. Aliquotation and storage at -20°C is recommended.

IBIAN Wash Buffer I and **II** charged with ethanol should be appropriately sealed and stored at room temperature.

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

IBIAN guarantees the correct function of the **IBIAN® DNA Blood Mini Kit** for applications as described in the manual. All components of the **IBIAN® DNA Blood Mini Kit** were tested against predetermined specifications to ensure consistent product quality.

If you have any questions or problems regarding any aspects of **IBIAN® DNA Blood Mini Kit** or other IBIAN products, please do not hesitate to contact us.

For technical support or further information please contact:
+34 976901645

Intended Use

The **IBIAN® DNA Blood Mini Kit**, is the ideal tool for a fast and convenient manual isolation and purification of genomic DNA from max. 200 µl fresh, frozen or old human and from max. 100 µl mammalian blood as well from buffy coat (max. 30 µl) or non mammalian blood (max. 25 µl). For reproducible and high yields an appropriate sample storage is essential. The purified DNA can be used for in-vitro diagnostic analysis.

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

The 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 professional users 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 modification of DNA followed by signal detection or amplification. Any diagnostic results generated 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.

Product Use Limitation

The kit is neither validated for the isolation of genomic DNA from serum or plasma, nor from bacteria, fungi, parasites, or purification of RNA.

The application of the kit for isolation and purification of viral DNA has not been evaluated. The kits applicability for cultured or isolated cells, tissue, stool sample, swabs, dried blood stains, or cell free body fluids, like cerebrospinal fluid, synovial fluid, and urine, also have not been validated. When changing the starting material or the flow trace, no guarantee in operability is issued.

The user is responsible to validate the performance of the IBIAN kits for any particular use, since the performance characteristics of our kits have not been validated for any specific application. IBIAN kits may be used in clinical diagnostic laboratory systems after the laboratory has validated the complete diagnostic system as required by CLIA' 88 regulations in the U.S. or equivalents in other countries.

All products sold by IBIAN are subjected to extensive quality control procedures. Any problems should be reported immediately.

The chemicals and plastic parts are for laboratory use only, they have to be stored in the laboratory and must not be used for other purposes than intended.

The kit contents are unfit for consumption.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. Heed the legal requirements for working with biological material!

If the buffer bottles are damaged or leaking, wear gloves and protective goggles when discarding the bottles in order to avoid any injuries.

IBIAN has not tested the liquid waste generated by the **IBIAN® DNA 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.

Below European Community risk and safety phrases for the components of the **IBIAN® DNA Blood Mini Kit** to which they apply, are listed.

IBIAN Lysis Buffer HL:



Xn contains: guanidine hydrochlorid
harmful
(R36, S2, S24)



Xi , F

IBIAN Binding Buffer HL:

highly flammable, irritant
(R11, 36, 67, S2, 7,16, S24/25/26)

Proteinase K:



Xn
harmful
(R36/37/38)

- R:11:** Highly flammable
- R36:** irritating to eyes
- R37:** irritating to respiratory system
- R38:** irritating to skin
- R67:** Vapours may cause drowsiness and dizziness

- S2:** Keep out of the reach of children
- S7:** Keep container tightly closed
- S16:** Keep away from sources of ignition - No smoking
- S22:** Do not breath dust
- S24:** Avoid contact with skin
- S25:** Avoid contact with eyes
- S26:** in case of contact with eyes, rinse immediately with plenty of water seek medical advice

Product Characteristic of the IBIAN® DNA Blood Mini Kit

starting material	yield	time	ratio
1 - 200 µl fresh, frozen or old human or other mammalian whole blood (EDTA, Citrat), 1 - 200 µl cerebrospinal fluid 1 - 30 µl buffy coat, 1 - 25 µl fresh, frozen or old non mammalian blood 1 - 20 µl bone marrow	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. 25min	$A_{260} : A_{280}$ 1.7 - 2.0

The **IBIAN DNA 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 purification procedure is rapid and requires neither phenol/ chloroform extraction, 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 IBIAN offers the **IBIAN® DNA Blood Mini HTS 96 Kit** for use in a centrifuge, on a vacuum manifold and on common laboratory automated workstations.

Principle and procedure

The **IBIAN® DNA Blood Mini Kit** procedure comprises following steps:

1. lysis of sample material
2. binding the genomic DNA to the membrane of a **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 (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, they could clog the **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 **IBIAN®** 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.

CSF and Bone Marrow:

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

Procedure

Lysis

Samples are lysed under anti-chaotropic conditions at elevated temperatures. Lysis is performed in the presence of **IBIAN Lysis Buffer HL** and **Proteinase K**.

Binding genomic DNA

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

Removing residual contaminations

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

Elution

Genomic DNA is eluted from the **Spin Filter** using 30 - 200 µl **IBIAN Elution Buffer D**. The eluted DNA is ready for use in different downstream applications. Eluted DNA stored at 4 – 8°C is stable for 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 using the **IBIAN® DNA Blood Mini Kit** procedure, depends on the sample type and the number of cells in the sample (depending from the patients age and health situation, sample source, transport conditions, storage, and age of the sample).

Typically, a 200 µl sample of whole blood cells . Samples with elevated white blood cell (WBC counts, ranging from 3×10^6 to 1×10^7 cells/ml) from a healthy individual will yield 3–10 µg of DNA.

For most whole blood samples, a single elution with 200 µl **IBIAN® Elution Buffer** 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

After receiving the kit, check the kit components for damage. If buffer bottles are damaged, contact the **IBIAN** Technical Services or your local distributor. In case of liquid spillage, refer to “Safety Information’s” (page 6). Do not use damaged kit components, since their use may lead to poor kit performance.

- always change pipet tips between liquid transfer. To avoid cross-contamination, we recommend the use of aerosol-barrier pipet tips
- all centrifugation steps are carried out at room temperature
- when working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles
- discard gloves if they become contaminated
- do not combine components of different kits unless the lot numbers are identical
- avoid microbial contamination of the kit reagents
- to minimize the risk of infections from potentially infectious material, we recommend working under laminar air-flow until the samples are lysed
- this kit should only be used by trained personal

Preparing reagents and buffers

1 adjust the thermomixer to 56°C.

2. warm up the needed amount of **IBIAN Elution Buffer D** to 56°C
(100 - 200 µl **IBIAN Elution Buffer D** are needed per sample).

3. label the needed amount of 2,0 ml **Receiver Tubes**

4. label the needed amount of 1.5 ml **Receiver Tubes** (per sample: 1 Receiver Tube)

5. add the needed µl dd H₂O to reaction tube with **Proteinase K** (see below). Vortex for 5 s;

6. add the needed amount of ethanol to the **IBIAN Wash Buffer I and II**.

7. *for 3 or 10 DNA-extractions:*

Add 250 µl dd H₂O to **Proteinase K**, mix thoroughly (vortex 5s)

and store at -20°C . IBIAN Wash Buffer I and II are ready to use

for 50 DNA-extractions:

Add 1,1 ml dd H₂O to **Proteinase K**, mix thoroughly (vortex 5s) and store at -20°C

Add 30 ml of 96 - 100 % ethanol to **IBIAN Wash Buffer I**

Add 42 ml of 96 - 100 % ethanol to **IBIAN Wash Buffer II**, mix thoroughly and always keep the bottle firmly closed!

for 250 DNA-extractions

Add 1,1 ml dd H₂O to **Proteinase K**, mix thoroughly (vortex 5s) and store at -20°C !

Add 80 ml of 96 - 100 % ethanol to **IBIAN Wash Buffer I**

Add 105 ml of 96 - 100 % ethanol to **IBIAN Wash Buffer II**, mix thoroughly and always keep the bottle firmly closed!!

Reagents and equipment to be supplied by user


When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

- 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 H₂O
- 96 - 100 % ethanol
- 1 x PBS (optional)

Important indications

1. Process only as much blood samples as the microcentrifuge allows to process.
2. **Blood sample and Buffers should be thoroughly mixed and should have room temperature ($18 - 25^{\circ}\text{C}$).**
3. The elution can be done by using lower amount of **IBIAN Elution Buffer D**. This may result in a higher concentration of DNA. But pay attention that minimum volume for elution is 50 μl , but this will reduce the yield. Elution volume between 2 x 50 μl up to 200 μl will realize comparable results.
4. **The eluated DNA volume can be lower than the added IBIAN Elution Buffer D volume. IBIAN Elution Buffer D should be preheated to 56°C .**
5. **The IBIAN Elution Buffer D doesn't contain EDTA.**
6. The yield can be increased, if the incubation time with preheated **IBIAN Elution Buffer D** will be prolonged.
7. Old blood samples often contains coagulants, if coagulants or cryoprecipitates (formed during thawing of frozen samples) are visible avoid aspirating them, they could clog the Spin Filter membrane.

Scheme

 <p>genomic DNA</p>	<p>Please read protocols prior the start of the preparation carefully</p> <hr/> <p>Transfer max. 200 μl of the blood into a 1.5 ml Receiver Tube</p> <p>Add 200 μl IBIAN Lysis Buffer HL Incubate for 3 min at 56°C while continuously shaking Add 20 μl Proteinase K and mix 5 times by pipetting up and or use the Thermomixer</p> <p>Incubate for 5 min at 56°C while continuously shaking</p> <p>Add 200 μl IBIAN Binding Buffer HL and mix by pipetting up and down four times or vortexing</p> <p>Take a RTA Spin Filter Set Transfer lysate onto RTA Spin Filter Centrifuge for 2 min at 13,000 x g Discard the filtrate and the RTA Receiver Tube</p> <p>Transfer the RTA Spin Filter in a new RTA Receiver Tube Add 500 μl IBIAN Wash Buffer I Centrifuge for 1 min at 13,000 x g Discard the filtrate and the RTA Receiver Tube</p> <p>Place RTA Spin Filter to a new 2.0 ml RTA Receiver Tube Add 700 μl IBIAN Wash Buffer II Centrifuge for 1 min at 13,000 x g 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 13,000 x g for ethanol removal</p> <p>Place the RTA Spin Filter into a 1.5 ml Receiver Tube Add 100 - 200 μl of IBIAN Elution Buffer D (preheated to 56°C) Incubate for 1 min at room temperature Centrifuge for 1 min at 8,000 x g Discard RTA Spin Filter Close the 1.5 ml Receiver Tube and store the DNA sample at 4 °C</p>
---	--

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

For isolation of genomic DNA from up to 200 µl whole blood or from up to 30 µl buffy coat

Please read the instructions carefully and conduct the prepared procedure.

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

1. Transfer 1 - 200 µl whole blood or 1 – 30 µl buffy coat into a 1.5 ml reaction tube. If sample volume is lower than 200 µl, equilibrate with 1 x PBS Buffer or distilled water to 200 µl.

2. Add 200 µl IBIAN Lysis Buffer HL and incubate for 3 min at 56°C while continuously shaking. Add 20 µl Proteinase K and mix by pipeting up and down (5 times) or use the Thermomixer.

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

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

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

5. Centrifuge for 2 min at 13.000 x g. Discard the filtrate and place the **RTA Spin Filter** in a new 2.0 ml RTA Receiver Tube.

6. Add 500 µl IBIAN Wash Buffer I to the **RTA Spin Filter**. Close the **RTA Spin Filter**. Centrifuge for 1 min at 13.000 x g. Discard the filtrate and place the **RTA Spin Filter** in a new **RTA Receiver Tube**.

7. Add 700 µl IBIAN Wash Buffer II and centrifuge for 1 min at 13.000 x g. Discard the filtrate and place the **RTA Spin Filter** in a new **RTA Receiver Tube**.

8. Add 700 µl IBIAN Wash Buffer II and centrifuge for 1 min at 13.000 x g. Discard the filtrate.

9. 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.

10. Place the RTA Spin Filter in a new 1.5 ml **Receiver Tube**. Add 200 µl of the preheated (56°C) **IBIAN Elution Buffer D**. Incubate at room temperature for 1 min.

11. Centrifuge at 8.000 x g for 1 min. Discard the RTA Spin Filter.

Note: The DNA can also be eluted with a lower volume of IBIAN Elution Buffer D depends on the expected yield of genomic DNA). But pay attention that 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 IBIAN Elution Buffer can be increased.

Protocol 2: DNA Isolation from non mammalian blood sample material

For isolation of genomic DNA from up to 25 µl non mammalian blood sample

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 IBIAN Elution Buffer D into an 2.0 ml Receiver Tube (not included in the kit) and place the tube at 56°C.

1. Transfer 1 - 25 µl whole blood into a 1.5 ml reaction tube. If sample volume is lower than 200 µl, equilibrate with 1 x PBS Buffer to 200 µl.

2. Add 200 µl IBIAN Lysis Buffer HL and incubate for 3 min at 56°C while continuously shaking. Add **20 µl Proteinase K and mix by pipetting up and down (5 times) or use the Thermomixer.**

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

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

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

5. Centrifuge for 2 min at 13.000 x g. Discard the filtrate and place the **RTA Spin Filter** in a new 2.0 ml RTA Receiver Tube.

6. Add 500 µl IBIAN Wash Buffer I to the **RTA Spin Filter**. Close the **RTA Spin Filter**. Centrifuge for 1 min at 13.000 x g. Discard the filtrate and place the **RTA Spin Filter** in a new **RTA Receiver Tube**.

7. Add 700 µl IBIAN Wash Buffer II and centrifuge for 1 min at 13.000 x g. Discard the filtrate and place the **RTA Spin Filter** in a new **RTA Receiver Tube**.

8. Add 700 µl IBIAN Wash Buffer II and centrifuge for 1 min at 13.000 x g. Discard the filtrate.

9. 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.

10. Place the RTA Spin Filter in a new 1.5 ml **Receiver Tube**. Add 200 µl of the preheated (56°C) **IBIAN Elution Buffer D**. Incubate at room temperature for 1 min.

11. Centrifuge at 8.000 x g for 1 min. Discard the RTA Spin Filter.

Note: The DNA can also be eluted with a lower volume of IBIAN Elution Buffer D depends on the expected yield of genomic DNA). But pay attention that 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 IBIAN Elution Buffer can be increased.

Protocol 3: DNA isolation from CFS and 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 IBIAN Elution Buffer D into an 2.0 ml Receiver Tube (not included in the kit) and place the tube at 56°C.

Preparation of the starting material:

Fresh material:

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

Dried material (for example on hematological slides):

- Moisten the dried material with a drop of PBS.
- Add 180 µl PBS to a 1.5 ml Receiver Tube (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.

I. Sample Lysis

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

2. Add 200 µl IBIAN Lysis Buffer HL and incubate for 3 min at 56°C while continuously shaking. Add 20 µl **Proteinase K** and mix by pipeting up and down (5 times) or use the Thermomixer

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

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

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

Proceed as described in protocol 1 steps 4 – 11.

Troubleshooting

Problem	Cause	Comments and suggestions
Low amount of DNA	<ul style="list-style-type: none"> insufficient lysis Inefficient binding of DNA to the membrane incomplete elution low DNA-concentration in the sample 	<ul style="list-style-type: none"> increase lysis time with IBIAN Lysis Buffer HL. reduce amount of starting material. continuously shaking improves lysis efficiency. overloading Spin Filter reduces yield <ul style="list-style-type: none"> - use correct amount of IBIAN Binding Buffer HL - mix sample with IBIAN Binding Buffer HL by pipetting up and down 4-5 times or by vortexing (5 sec) prior to transfer the sample onto the Spin Filter increase incubation time with preheated IBIAN Elution Buffer D to 5 - 10 min eluting twice with each 100 µl IBIAN Elution Buffer D use higher volume of IBIAN Elution Buffer D. elute the DNA with lower volume of IBIAN Elution Buffer D
Spin Filter surface tints yellow	<ul style="list-style-type: none"> insufficient lysis inefficient washing old material 	<ul style="list-style-type: none"> see above wash again with IBIAN Wash Buffer II perform isolation as described in protocol 2
Degraded or sheared DNA	<ul style="list-style-type: none"> incorrect storage of starting material old material 	<ul style="list-style-type: none"> ensure the sample is harvested and stored as described on page 8 avoid repeated thawing and freezing of the material old material often contains degraded DNA
Problems with subsequent applications (e.g. in PCR)	<ul style="list-style-type: none"> ethanol in the eluted DNA salt in the eluat 	<ul style="list-style-type: none"> verify if the recommended centrifugation time has been adhered increase centrifugation time for the elimination of ethanol if necessary Wash Buffer should be stored at and used at RT verify Wash Buffer on the precipitation of salt. In possibly precipitations dissolve this by careful warming up to 30°C
Clogged Spin Filter	<ul style="list-style-type: none"> Incorrect storage of starting material Insufficient lysis 	<ul style="list-style-type: none"> perform isolation as described in protocol 2 increase lysis time with IBIAN Lysis Buffer HL increase centrifugation time and/or speed reduce amount of starting material

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 lyses conditions of blood differs depending on the species. Also remember that non-mammalian blood contains erythrocytes with nuclei. So for special applications adaptation of starting volumes and lyses time may be recommended.

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 overdrying 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, transport, storage, and age. Various different primary tubes and anticoagulants (except heparin) can be used to collect blood samples for the procedure.