IBIAN® Tissue Mini kit

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IBIAN® Tissue Mini kit

For DNA extractions from 0.5 - 40 mg tissue sample, rodent tail and paraffine embedded material

The **IBIAN® Tissue Mini kit** is the ideal tool for purification of DNA from small amounts of various human and animal tissues (e.g. muscle, liver, heart and brain) **in less than 20 min.** This system is particularly efficient for the isolation of DNA from biopsy material and paraffine embedded tissues. No toxic or hazardous chemicals like chaotropic components or phenol/chloroform are used.
Due to the high purity, the isolated genomic DNA is ready to use for a broad panel of downstream applications:

- PCR*
- RFLP-Analysis
- Restriction Enzyme Digestion
- HLA Typing
- Sequencing*
- Cloning*
- Southern Blot

Name	Amount of starting material	Yield	Time for preparation
IBIAN® Tissue Mini kit	0,5 – 40 mg tissue sample up to 1.2 cm rodent tail micro and fine needle biopsy material	up to 50 µg depends on type and amount of starting material	15 min after lysis **Ratio** A_{260} : A_{280} 1.7 – 2.0

Reagents and equipment to be supplied by user

- Microcentrifuge
- Eppendorf Thermomixer (for 52°C)
- Reaction tubes (1.5ml or 2.0ml)
- dd H_2O
- 99,8% Ethanol
- Octane (optional for deparaffination)
- optional: RNAse A (10mg/ml)

*) The PCR method is covered by U.S. Patents 4,683,195 and 4,683,202 owned by Hoffmann-LaRoche Inc. The purchase of the **IBIAN® Tissue Mini kit** cannot be construed as an authorization or implicit licence to practice PCR under any patents held by Hoffmann-LaRoche Inc.

Safety precautions

IBIAN Lysis Buffer G and IBIAN Binding Buffer T contain a detergent wich is an irritant. Take appropriate safety measures. Always wear gloves while handling these reagents and avoid any skin contact!
Below is listed European Community risk and safety phrases for the components of the IBIAN® Tissue Mini kit to which they apply.

IBIAN Binding Buffer T: Highly flammable, Irritant (R11-36-67, S2-7-16-24/25-26)
Proteinase K: Harmful (R36/37/38-42, S2-22-24-26-36/37)

Storage conditions

The IBIAN® Tissue Mini kit should be stored dry, at room temperature (18 – 25°C) and is stable for at least 12 months under these conditions. Proteinase K must be stored at – 20°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 careful warming.

Function testing and technical assistance

IBIAN guarantees the correct function of the IBIAN® Tissue Mini kit for applications as described in the manual. The components of each IBIAN® Tissue Mini kit were tested by isolation of genomic DNA from 10 mg frozen mouse liver sample.
We reserve the right to change or modify our products to enhance their performance and design.
If you have any questions or problems regarding any aspects of IBIAN® Tissue Mini kit or other IBIAN products, please do not hesitate to contact us. For technical support or further information in Spain please dial +34-976 141 693 or +34-976 901 645. For other countries please contact your local distributor.

Product use and warranty

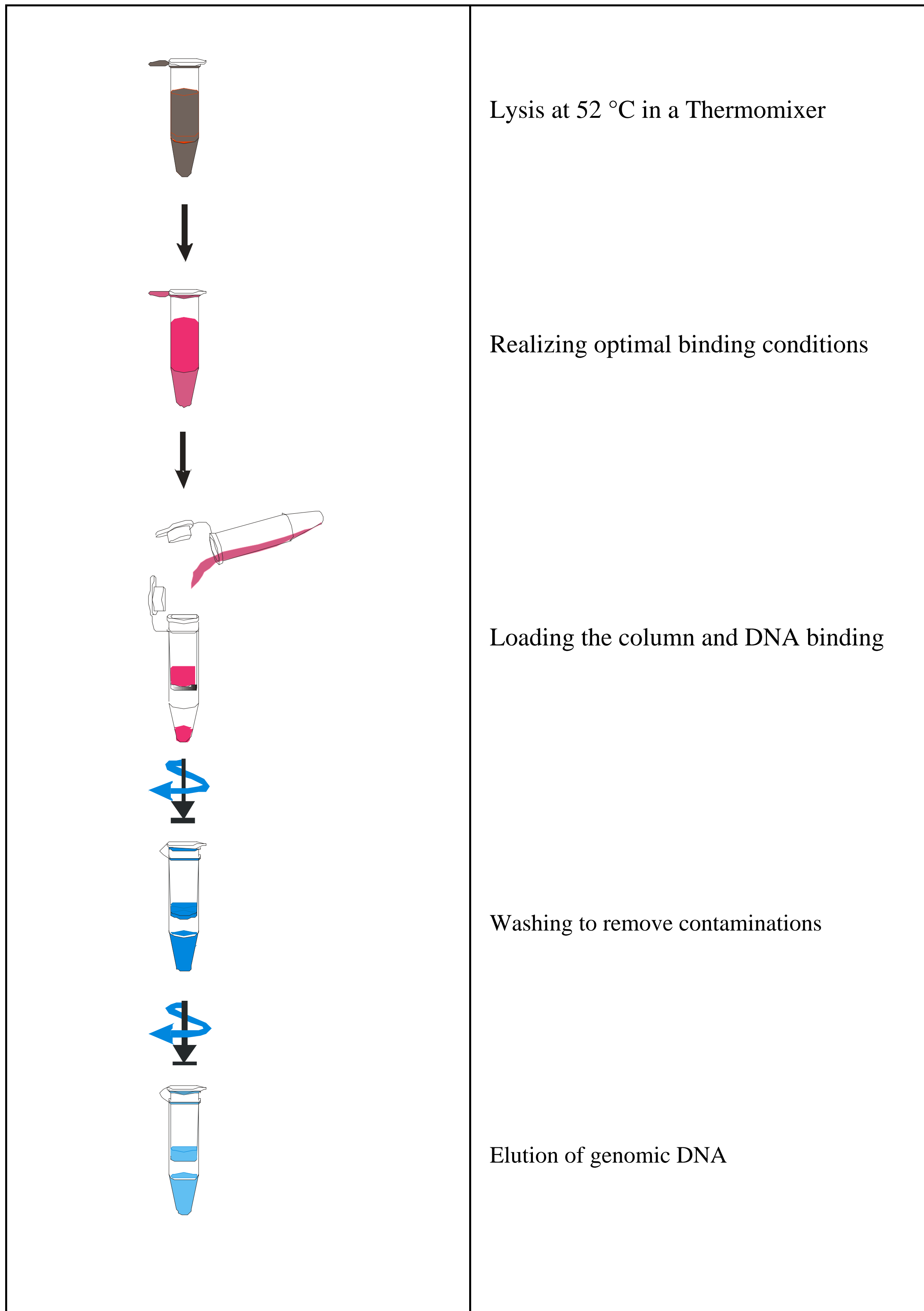
The user is responsible to validate the performance of the IBIAN kits for any particular use, since the performance characteristics of our kits have not been validated for any specific application. IBIAN kits may be used in clinical diagnostic laboratory systems after the laboratory has validated the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

Kit components (storage at room temperature)

Important: ◆ **Store lyophilized Proteinase K at 2 - 8 °C ;**
Store diluted Proteinase K at – 20 °C, but repeated freezing and thawing will reduced the activity dramatically. Dividing the Proteinase K into aliquots and storage at – 20°C is recommended.

	3 DNA extractions	**10 DNA extractions**	**50 DNA extractions**	**250 DNA extractions**
IBIAN Lysis Buffer G	2 ml	3 x 2 ml	30 ml	120 ml
IBIAN Binding Buffer T	2 ml	2 x 2 ml	15 ml	60 ml
Proteinase K	for 250 µl working solution	for 500 µl working solution	for 2 ml working solution	for 5 x 2 ml working solution
IBIAN Wash Buffer	15 ml (ready to use)	15 ml (ready to use)	18 ml (final volume 60 ml)	2 x 45 ml (final volume 2 x 150 ml)
IBIAN Elution Buffer D	2 ml	2 x 2 ml	30 ml	120 ml
Spin Filter	3	10	50	250
2.0 ml Receiver Tubes	3	10	50	250
1.5 ml Receiver Tubes	3	10	50	250
Manual	1	1	1	1
Initial steps	• Add 250 µl dd H_2O to the tube Proteinase K, mix thoroughly and store the tube at -20°C! • Incubate the needed amount of IBIAN Elution Buffer D at 52°C.	• Add 500 µl dd H_2O to the tube Proteinase K, mix thoroughly and store the tube at -20°C! • Incubate the needed amount of IBIAN Elution Buffer D at 52°C.	• Add 42 ml of 96-100% Ethanol to the bottle Wash Buffer, mix thoroughly and keep the bottle always firmly closed ! • Add 2 ml dd H_2O to the tube Proteinase K, mix thoroughly and store the tube at -20°C! • Incubate the needed amount of IBIAN Elution Buffer D at 52°C.	• Add 105 ml of 96-100% Ethanol to each bottle Wash Buffer, mix thoroughly and keep the bottle always firmly closed ! • Add 2 ml dd H_2O to each tube Proteinase K, mix thoroughly and store the tube at-20°C ! • Incubate the needed amount of IBIAN Elution Buffer D at 52°C.

DNA Extraction from Tissue



Protocol 1: DNA Extraction from 0.5 - 40 mg tissue and rodent tail (up to 1.2 cm)

Important: ◆ Prewarm the IBIAN Elution Buffer D to 52°C (e.g. transfer the needed volume into an Eppendorf tube and place the tube at the appropriate temperature)
When using liver tissue please take not more than 20 mg !

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1. Lysis of the starting material

Transfer the starting material into a 1.5 ml tube.
A mechanical grinding or a cutting of the material will increase the lysis efficiency.
Add 400 µl IBIAN Lysis Buffer G and 40 µl Proteinase K and vortex thoroughly.
Incubate at 52°C up to the lysis is complete (incubation in a thermomixer under continuous shaking is recommended).

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2. Realizing optimal binding conditions

Centrifuge for 2 min at maximum speed to spin down all non lysed material.
Transfer the supernatant into a new 1.5 ml tube.

Attention: ◆ To remove RNA from the sample (if necessary) add 40 µl of RNase A (10mg/ml), vortex shortly and incubate for 5 min at RT

Add 200 µl IBIAN Binding Buffer T and vortex for 10 sec.

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3. DNA Binding

Place a spin column into a 2.0 ml Receiver Tube.
Transfer the suspension onto the Spin Column and incubate for 1 min.
Centrifuge at 12,000 rpm for 2 min. Discard the filtrate and place the column again into the Receiver Tube.

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4. Washing

Add **550 µl IBIAN Wash Buffer** and centrifuge at 12,000 rpm for 1min. Discard the filtrate, place the column again into the Receiver Tube and repeat the washing step once. Discard the filtrate, put the column back into the Receiver Tube and remove the residual ethanol by final centrifugation for 2 min at 12,000 rpm.

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5. Elution of the DNA

Place the Spin Column into a 1.5 ml Receiver Tube and add 200 µl of the prewarmed IBIAN Elution
Buffer D. Incubate for 3min. Centrifuge for 2 min at 10,000 rpm.

Note: ◆ The DNA can also be eluted with a lower or a higher volume of IBIAN Elution Buffer D (depends on the expected yield of genomic DNA). But pay attention that minimum volume for the elution is **50 µl**.
If quite large amount of DNA is expected, the volume of elution can be increased.

Protocol 2: DNA Extraction from paraffine embedded tissue

1. Transfer the starting material into a 1.5 ml tube.

Add 1 ml Octane and vortex carefully to dissolve the paraffine. Follow the dissolution until the tissue sample looks transparent (while paraffin is still white)

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2. Centrifugation for 2 min at maximum speed to pellet down the tissue sample.

Discard the supernatant very careful.
This step should be repeated if any paraffine is still remained in the sample.

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3. Add 0.5 ml 96 % Ethanol to the tissue sample and mix the tube thoroughly.

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4. Short centrifugation and removing of the ethanol by aspiration with pipette.
Then incubate the open tube at 52°C to evaporate the residual ethanol.

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5. Lysis of the starting material

A mechanical grinding or a cutting of the deparaffined material will increase the lysis efficiency.
Add 400 µl IBIAN Lysis Buffer G and 40 µl Proteinase K to the sample and vortex thoroughly.
Incubate at 52°C up to the lysis is complete (incubation in an thermomixer under continuous shaking is recommended).

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6. Realizing optimal binding conditions

Centrifuge for 2 min at max. speed to spin down all non lysed material.
Transfer the supernatant into a new 1.5 ml tube.

Attention: ◆ To remove RNA from the sample (if necessary) add 40 µl of RNase A (10mg/ml), vortex shortly and incubate for 5 min at RT.

Add 200 µl IBIAN Binding Buffer T and vortex for 10 sec.

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7. DNA Binding

Place a spin column into a 2.0 ml Receiver Tube.
Transfer the suspension onto the Spin Column and incubate for 1 min.
Centrifuge at 12,000 rpm for 2 min. Discard the filtrate and place the column again into the Receiver Tube.

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8. Washing

Add 550 µl IBIAN Wash Buffer and centrifuge at 12,000 rpm for 1min. Discard the filtrate, place the column again into the Receiver Tube and repeat the washing step once. Discard the filtrate, put the column back into the Receiver Tube and remove the residual ethanol by final centrifugation for 2 min at 12,000 rpm.

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9. Elution of the DNA

Place the Spin Column into a 1.5 ml Receiver Tube and add 50 µl of the prewarmed IBIAN Elution Buffer D. Incubate for 3 min. Centrifuge for 2 min at 10,000 rpm.

Note: ◆ The DNA can also be eluted with a higher 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.

Protocol 3: DNA isolation from 10 – 10^7 eucaryotic cells / cell pellets

For the isolation and purification of DNA from small amounts of various human and animal tissues (e.g. muscle, liver, heart and brain), biopsy material, rodent tail and paraffin embedded tissues and eycaryotic cells.

Please read the instructions carefully and conduct the prepared procedure!

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1. Add **400 µl IBIAN Lysis Buffer G** and **40 µl Proteinase K** to the washed cell pellet and vortex thoroughly. Transfer the complete mixture to a 1.5 ml **reaction tube**.

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2. Incubate the **reaction tube** at 52°C up to the lysis is complete under continuously **shaking. For material difficult to lyse we recommend to vortex the tube several times.**

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3. Centrifuge for 2 min at maximum speed to spin down non lysed material. Transfer the supernatant into a new 1.5 ml tube.

Attention: ◆ To remove RNA from the sample (if necessary) add **40 µl of RNase A** (10mg/ml), vortex shortly and incubate for 5 min at RT

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4. Add **200 µl IBIAN Binding Buffer T** and vortex for 10 sec.

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5. Place a **Spin Column** into a 2.0 ml **receiver tube**. Transfer the suspension onto the **spin filter** and incubate for 1 min. Close **Spin filter** and centrifuge at 13,000 x g for 2 min. Discard the filtrate and place the column again into the **receiver tube**.

Attention: ◆ Ensure that you have already added the recommended amount of ethanol to the Wash Buffer!

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6. Add **550 µl Wash Buffer**, close **spin filter** and centrifuge at 13,000 x g for 1min. Discard the filtrate, place the column again into the **receiver tube.**

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7. Repeat the washing step once. Discard the filtrate, put the column back into the **receiver tube** and remove the residual ethanol by final centrifugation for 2 min at 13,000 x g.

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8. Place the **spin column** into a 1.5 ml **receiver tube** and add **200 µl of the prewarmed IBIAN Elution Buffer D**. Incubate for 3 min at room temperature. Centrifuge for 2 min at 8,500 x g.

Note: ◆ The DNA can also be eluted with a lower or a higher volume of **IBIAN Elution Buffer D** (depends on the expected yield of genomic DNA). But pay attention that minimum volume for the elution is **50 µl**.
If quite large amount of DNA is expected, the volume of elution can be increased.

Troubleshooting

Problem/probable cause	Comments and suggestions
Clogged Spin-Filter • insufficient lysis and/ or too much starting material	Increase lysis time. Increase centrifugation speed. After lysis spin lysate to pellet unlysed material and continue with the protocol using the supernatant. Reduce amount of starting material.
Low amount of extracted DNA • insufficient lysis	Increase lysis time. Reduce amount of starting material. Overloading of Spin Filter reduces yield!
• incomplete elution	Prolong the incubation time with IBIAN Elution Buffer D to 5-10 min or repeat elution step once again. Take higher volume of IBIAN Elution Buffer D.
• Insufficient mixing with IBIAN Binding Buffer T	Mix sample with IBIAN Binding Buffer T by pipetting or by vortexing prior to transfer the sample onto the spin column.
Low concentration of extracted DNA • to much Elution Buffer	Elute the DNA with lower volume of IBIAN Elution Buffer D.
Degraded or sheared DNA • incorrect storage of starting material	Ensure that the starting material is frozen immediately in liquid N_2 or in minimum at –20°C and is stored continuously at –80°C! Avoid thawing of the material.
• Old material	Old material often contains degraded DNA.
RNA contaminations of extracted DNA.	RNAase A digestion