

# IBIAN<sup>®</sup> DNA Plant 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](mailto:info@ibiantech.com)**



# IBIAN<sup>®</sup> DNA Plant Mini Kit

For DNA extractions from plant material (up to 100 mg wet weight)

The **IBIAN<sup>®</sup> DNA Plant Mini Kit** allows rapid and efficient isolation of high quality genomic DNA from a wide variety of plant species (fresh, frozen or dried plant material, for instance leaves, roots, fruits or seeds). The **IBIAN<sup>®</sup> DNA Plant Mini Kit** combines the lysis of starting material with the very efficient binding of genomic DNA onto a spin filter surface without chaotropic ions.

The isolation protocol as well as all buffers are optimized to provide high yield and purity of the isolated genomic DNA. The “hands-on time“ necessary for the whole procedure is reduced to minimum. Up to 100 mg of fresh (wet weight) plant material can be processed in about 20 minutes after lysis.

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
- Sequencing\*
- Cloning\*
- Southern Blot

Name	Amount of starting material	Yield	Time for preparation
<b>IBIAN<sup>®</sup> DNA Plant Mini Kit</b>	up to 100 mg of plant material	up to 50 µg depends on amount and kind of starting material	About 20 minutes after lysis
			<b>Ratio</b>
			$A_{260} : A_{280}$ 1.6 – 2.0

## Reagents and equipment to be supplied by user

- Microcentrifuge
- Eppendorf Thermomixer (for 65°C)
- Reaction tubes (1.5ml)
- dd H<sub>2</sub>O
- 99,8% ethanol
- optional RNase A (10 mg/ml; optional)

\*) 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<sup>®</sup> DNA Plant Mini Kit** cannot be construed as an authorization or implicit licence to practice PCR under any patents held by Hoffmann-LaRoche Inc.

## **Safety precautions**

Take appropriate safety measures. Always wear gloves while handling these reagents and avoid any skin contact! IBIAN Wash Buffer I contains a chaotropic component.

Below is listed European Community risk and safety phrases for the components of the IBIAN<sup>®</sup> DNA Plant Mini Kit to which they apply.

**IBIAN Lysis Buffer P:** Irritant (R36, S2-24)

**IBIAN Binding Buffer P:** 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)

**IBIAN Wash Buffer I:** Harmful (R20/21/22-32, S2-13)

## **Storage conditions**

The IBIAN<sup>®</sup> DNA Plant Mini Kit should be stored dry, at room temperature (18 – 25°C). It 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<sup>®</sup> DNA Plant Mini Kit for applications as described in the manual. The components of each IBIAN<sup>®</sup> DNA Plant Mini Kit were tested by isolation of genomic DNA from 40 mg frozen plant material.

We reserve the right to change or modify our products to enhance its performance and design.

If you have any questions or problems regarding any aspects of IBIAN<sup>®</sup> DNA Plant Mini Kit or other IBIAN products, please do not hesitate to contact us. For technical support or further information in Spain please dial ++34 976 901 645 or ++34 976 141 693. For other countries please contact your local distributor.

## **Product use and warranty**

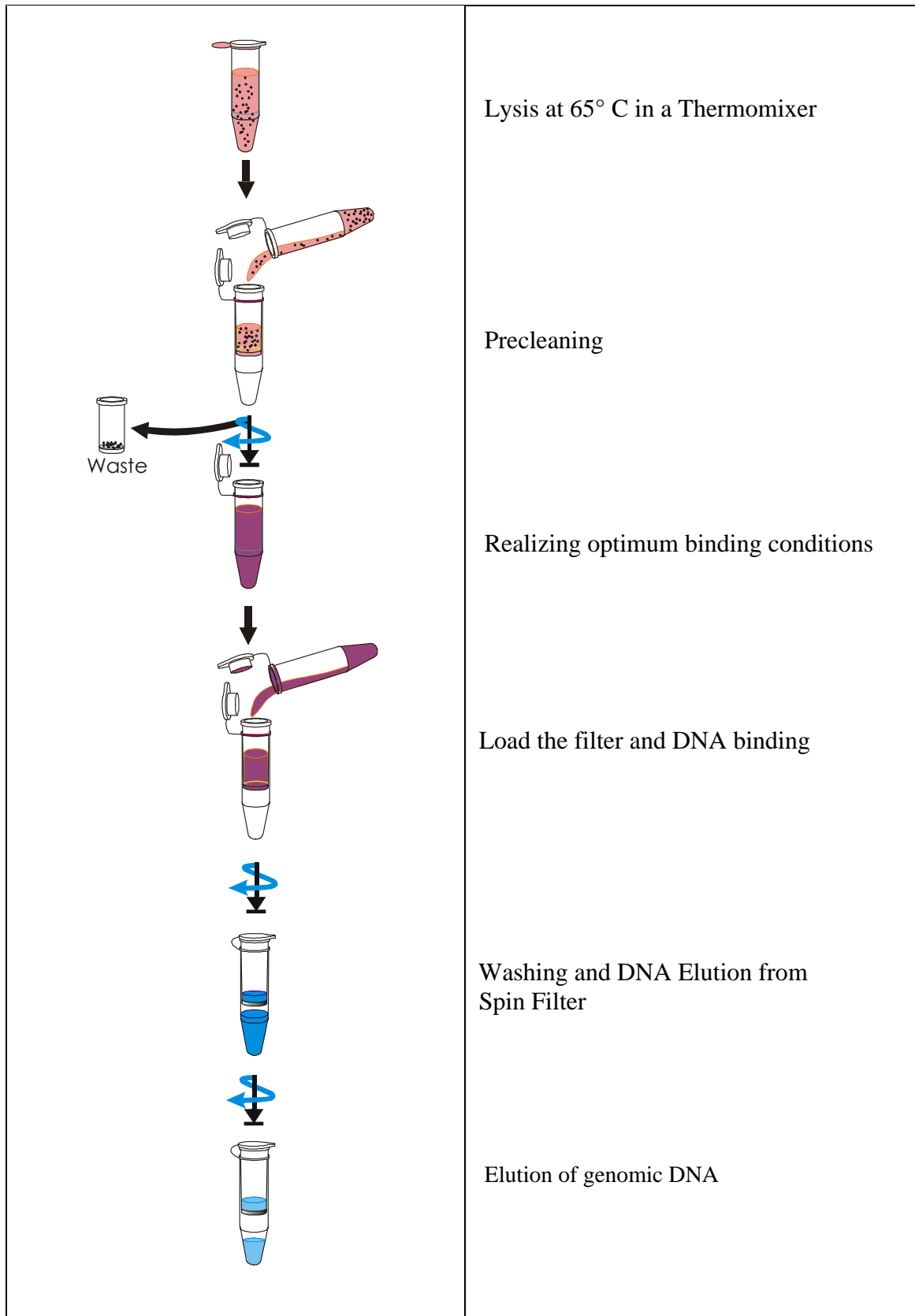
The user is responsible to validate the performance of the IBIAN kits for any particular use, since the performance characteristics of our kits have not been validated for any specific application. IBIAN kits may be used in clinical diagnostic laboratory systems after the laboratory has validated the 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
<b>IBIAN Lysis Buffer P</b>	2 ml	3 x 2 ml	30 ml	120 ml
<b>IBIAN Binding Buffer P</b>	2 ml	2 x 2 ml	15 ml	60ml
<b>Proteinase K</b>	for 250 µl working solution	for 250 µl working solution	for 1 ml working solution	for 5 x 1 ml working solution
<b>IBIAN Wash Buffer I</b>	15 ml (ready to use)	15 ml (ready to use)	30 ml (final volume 60 ml)	1 x 80 ml (final volume 160 ml)
<b>IBIAN Wash Buffer II</b>	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)
<b>IBIAN Elution Buffer D</b>	2 ml	2 x 2 ml	15 ml	60 ml
<b>Spin Filter</b>	6	20	100	500
<b>2.0 ml Receiver Tubes</b>	6	20	100	500
<b>1.5 ml Receiver Tubes</b>	3	10	50	250
<b>Manual</b>	1	1	1	1
<b>Initial steps</b>	<ul style="list-style-type: none"> <li>• Add 250 µl dd H<sub>2</sub>O to the tube Proteinase K, mix thoroughly and store the tube at -20°C !</li> <li>• Incubate the needed amount of Elution Buffer D at 65°C in a Thermomixer.</li> </ul>	<ul style="list-style-type: none"> <li>• Add 250 µl dd H<sub>2</sub>O to the tube Proteinase K, mix thoroughly and store the tube at -20°C !</li> <li>• Incubate the needed amount of Elution Buffer D at 65°C in a Thermomixer.</li> </ul>	<ul style="list-style-type: none"> <li>• Add 30 ml of 96-100% Ethanol to the bottle IBIAN Wash Buffer I.</li> <li>• Add 42 ml of 96-100% Ethanol to the bottle IBIAN Wash Buffer II, mix thoroughly and store with tightly closed cap.</li> <li>• Add 1 ml dd H<sub>2</sub>O to the tube Proteinase K, mix thoroughly and store the tube at -20°C !</li> <li>• Incubate the needed amount of Elution Buffer D at 65°C in a Thermomixer.</li> </ul>	<ul style="list-style-type: none"> <li>• Add 80 ml of 96-100% Ethanol to the bottle IBIAN Wash Buffer I.</li> <li>• Add 105 ml of 96-100% Ethanol to each bottle IBIAN Wash Buffer II, mix thoroughly and store with tightly closed cap.</li> <li>• Add 1 ml dd H<sub>2</sub>O to the tube Proteinase K, mix thoroughly and store the tube at -20°C !</li> <li>• Incubate the needed amount of Elution Buffer D at 65°C in a Thermomixer.</li> </ul>

## Scheme - DNA Extraction



## **Protocol 1: DNA extraction from fresh or dried plant material**

**Important:** ♦ Prewarm the Elution Buffer D to 65°C (e.g. transfer the needed volume into an Eppendorf tube and place the tube at the appropriate temperature in a thermomixer).

---

### **1. Homogenization of the starting material**

Homogenize about 60 mg of dried or 100 mg of fresh starting material by a pestle under liquid N<sub>2</sub>. Commercially available equipment for homogenization also can be used.

**Note:** ♦ Use 120-180 mg of starting material if extraction from material containing more water (fruits, algae).

---

### **2. Lysis of the starting material**

Transfer the “plant powder” into a 1.5 ml reaction tube. Add 400 µl IBIAN Lysis Buffer P and 20 µl Proteinase K and vortex briefly. Incubation at 65°C for 30 min or longer (incubation in a thermomixer under continuous shaking is recommended). During incubation place a Spin Filter into a 2.0 ml Receiver Tube.

---

### **3. Filtration of Lysis Solution and realizing optimum binding conditions (optional RNA-digestion)**

Transfer of Lysis Solution onto the Spin Filter. Centrifuge for 1 min at 12,000 rpm. Remove Spin Filter.

**Note:** ♦ To remove RNA (if it is necessary) from the sample add 40 µl of RNase A to the filtrate (10 mg/ml), vortex briefly and incubate for 5 min at room temperature. Add 200 µl of IBIAN Binding Buffer P and vortex thoroughly.

---

### **4. DNA Binding**

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

---

### **5. Washing I**

Add 550 µl IBIAN Wash Buffer I and centrifuge at 12,000 rpm for 1min. Discard the filtrate, place the Spin Filter again into the 2.0 ml Receiver Tube.

---

### **6. Washing II**

Add 550 µl IBIAN Wash Buffer II and centrifuge at 12,000 rpm for 1min. Discard the filtrate, place the Spin Filter again into the Receiver Tube and repeat the washing step once again. Finally discard the filtrate and centrifuge for 2 min at 12,000 rpm (to remove residual ethanol).

---

### **7. Elution of the DNA**

Place the Spin Filter into a new 1.5 ml Receiver Tube and add 100 µl of the prewarmed Elution Buffer D. Incubate for 3 min. Centrifuge for 1 min at 10,000 rpm.

**Note:** ♦ The DNA can also be eluted with a lower or a higher volume of 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 (100-200 µl).

**To maximize the final yield we recommend a second elution step with the equal volume of Elution Buffer D.**

## **Protocol 2: genomic and bacterial DNA extraction from fresh or dried plant material**

**Important:** ♦ Prewarm the Elution Buffer D to 65°C (e.g. transfer the needed volume into an Eppendorf tube and place the tube at the appropriate temperature in a thermomixer).

---

### **1. Homogenization of the starting material**

Homogenize about 60 mg of dried or 100 mg of fresh starting material by a pestle under liquid N<sub>2</sub>. Commercially available equipment for homogenization also can be used.

**Note:** ♦ Use 120-180 mg of starting material if extraction from material containing more water (fruits, algae).

---

### **2. Lysis of the starting material**

Transfer the “plant powder” into a 1.5 ml reaction tube. Add 400 µl IBIAN Lysis Buffer P and 20 µl Lysozyme (10mg/ml stock solution) and vortex briefly. Incubate for 20 min at 37°C. Add now 20 µl Proteinase K and vortex briefly. Incubation at 65°C for 30 min or longer (incubation in a thermomixer under continuous shaking is recommended). During incubation place a Spin Filter into a 2.0 ml Receiver Tube.

---

### **3. Filtration of Lysis Solution and realizing optimum binding conditions (optional RNA-digestion)**

Transfer of Lysis Solution onto the Spin Filter. Centrifuge for 1 min at 12,000 rpm. Remove Spin Filter.

**Note:** ♦ To remove RNA (if it is necessary) from the sample add 40 µl of RNase A to the filtrate (10 mg/ml), vortex briefly and incubate for 5 min at room temperature.

Add 200 µl of IBIAN Binding Buffer P and vortex thoroughly.

---

### **4. DNA Binding**

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

---

### **5. Washing I**

Add 550 µl IBIAN Wash Buffer I and centrifuge at 12,000 rpm for 1min. Discard the filtrate, place the Spin Filter again into the 2.0 ml Receiver Tube.

---

### **6. Washing II**

Add 550 µl IBIAN Wash Buffer II and centrifuge at 12,000 rpm for 1min. Discard the filtrate, place the Spin Filter again into the Receiver Tube and repeat the washing step once again. Finally discard the filtrate and centrifuge for 2 min at 12,000 rpm (to remove residual ethanol).

---

### **7. Elution of the DNA**

Place the Spin Filter into a new 1.5 ml Receiver Tube and add 100 µl of the prewarmed Elution Buffer D. Incubate for 3 min. Centrifuge for 1 min at 10,000 rpm.

**Note:** ♦ The DNA can also be eluted with a lower or a higher volume of 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 (100-200 µl).

To maximize the final yield we recommend a second elution step with the equal volume of Elution Buffer D.

### Troubleshooting

<b>Problem/probable cause</b>	<b>Comments and suggestions</b>
<b>Clogged Spin-Filter</b> <ul style="list-style-type: none"> <li>Insufficient lysis and/ or too much starting material</li> </ul>	Increase lysis time. Increase centrifugation speed or time. Reduce amount of starting material.
<b>Low amount of extracted DNA</b> <ul style="list-style-type: none"> <li>Insufficient lysis</li> <li>Incomplete elution</li> <li>Insufficient mixing with IBIAN Binding Buffer P</li> </ul>	Increase lysis time. Reduce amount of starting material. Overloading of spin filter reduces yield!  Prolong the incubation time with Elution Buffer D to 5-10 min or repeat elution step once again. Take higher volume of Elution Buffer D.  Mix sample with IBIAN Binding Buffer P by pipetting or by vortexing prior to transfer the sample onto the Spin Filter.
<b>Low concentration of extracted DNA</b> <ul style="list-style-type: none"> <li>To much Elution Buffer D</li> </ul>	Elute the DNA with lower volume of Elution Buffer D.
<b>Degraded or shared DNA</b> <ul style="list-style-type: none"> <li>Incorrect storage of starting material</li> <li>Old material</li> </ul>	Ensure that the starting material is frozen immediately in liquid N <sub>2</sub> or in minimum at -20°C and is stored continuously at -80°C! Avoid thawing of the material.  Old material often contains degraded DNA.
<b>RNA contaminations of extracted DNA.</b>	RNase A digestion
<b>Total RNA does not perform well in downstream-applications (e.g. RT-PCR)</b> <ul style="list-style-type: none"> <li>Ethanol carryover during elution</li> <li>Salt carryover during elution</li> </ul>	Increase time for removing of ethanol.  Ensure that IBIAN Wash Buffer is at room temperature. Check up Wash Buffer for salt precipitates. If there are any precipitates, solve these precipitates by careful warming.