

# IBIAN<sup>®</sup> Plasmid Mini Kit



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## IBIAN<sup>®</sup> Plasmid Mini Kit

The **IBIAN<sup>®</sup> Plasmid Mini Kit** provides an extremely fast, efficient and low cost purification system for plasmid DNA from **0.5 ml to 2 ml** of bacterial cultures.

The **IBIAN<sup>®</sup> Plasmid Mini Kit** combines the alkaline lysis procedure for the isolation of plasmid DNA with the very efficient binding of plasmid DNA onto a Spin Filter surface.

The isolation protocol as well as all buffers are optimized to provide high yield and purity of the isolated plasmid DNA. The “hands-on time“ which is necessary for the whole procedure is reduced to a minimum.

Plasmid DNA purified by the **IBIAN<sup>®</sup> Plasmid Mini Kit** is immediately ready to use for a broad panel of downstream applications:

- PCR <sup>(1)</sup>
- Restriction Enzyme Digestion
- Labeling
- Cloning
- Sequencing

Name	Amount of starting material	Yield	Time for preparation
<b>IBIAN<sup>®</sup> Plasmid Mini Kit</b>	• 0.5 - 2 ml of bacterial cultures	• up to 20 µg (2 ml bacterial culture)	< 15 min
			<b>Ratio</b>
			A <sub>260</sub> : A <sub>280</sub> 1.8- 2.1

### Reagents and equipment to be supplied by user

- Microcentrifuge (12,000 –14,000 rpm)
- 99.8% Ethanol

1) 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> Plasmid Mini** cannot be construed as an authorization or implicit licence to practice PCR under any patents held by Hoffmann-LaRoche Inc.

## **Safety precautions**

All due care and attention should be exercised in handling the materials and reagents contained in the kit. Always wear gloves while handling these reagents and avoid any skin contact!

**In case of contact, immediately flush eyes or skin with a large amount of water.**

## **Storage conditions**

The **IBIAN<sup>®</sup> Plasmid Mini Kit** should be stored dry, at room temperature (18 – 25°C) and is stable for up to 12 months before use. Make sure that all components have room temperature. If there are any precipitates in the reagents provided, dissolve them by careful warming the reagents **up to RT**.

## **Function testing and technical assistance**

IBIAN<sup>®</sup> Technologies guarantees the correct function of the **IBIAN<sup>®</sup> Plasmid Mini Kit** for applications as described in the manual. The components of each **IBIAN<sup>®</sup> Plasmid Mini Kit** were tested by isolation of Plasmid pGEM DNA from 2.0 ml bacterial lysate and subsequent restriction endonuclease digestion, spectrophotometrical measurement and gel electrophoretical analysis.

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> Plasmid Mini Kit** or other **IBIAN<sup>®</sup>** 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<sup>®</sup>** kits for any particular use, since the performance characteristics of our kits have not been validated for any specific application. **IBIAN<sup>®</sup>** 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 Technologies** are subjected to extensive quality control and are warranted to perform as described when used correctly. Any problems should be reported immediately.

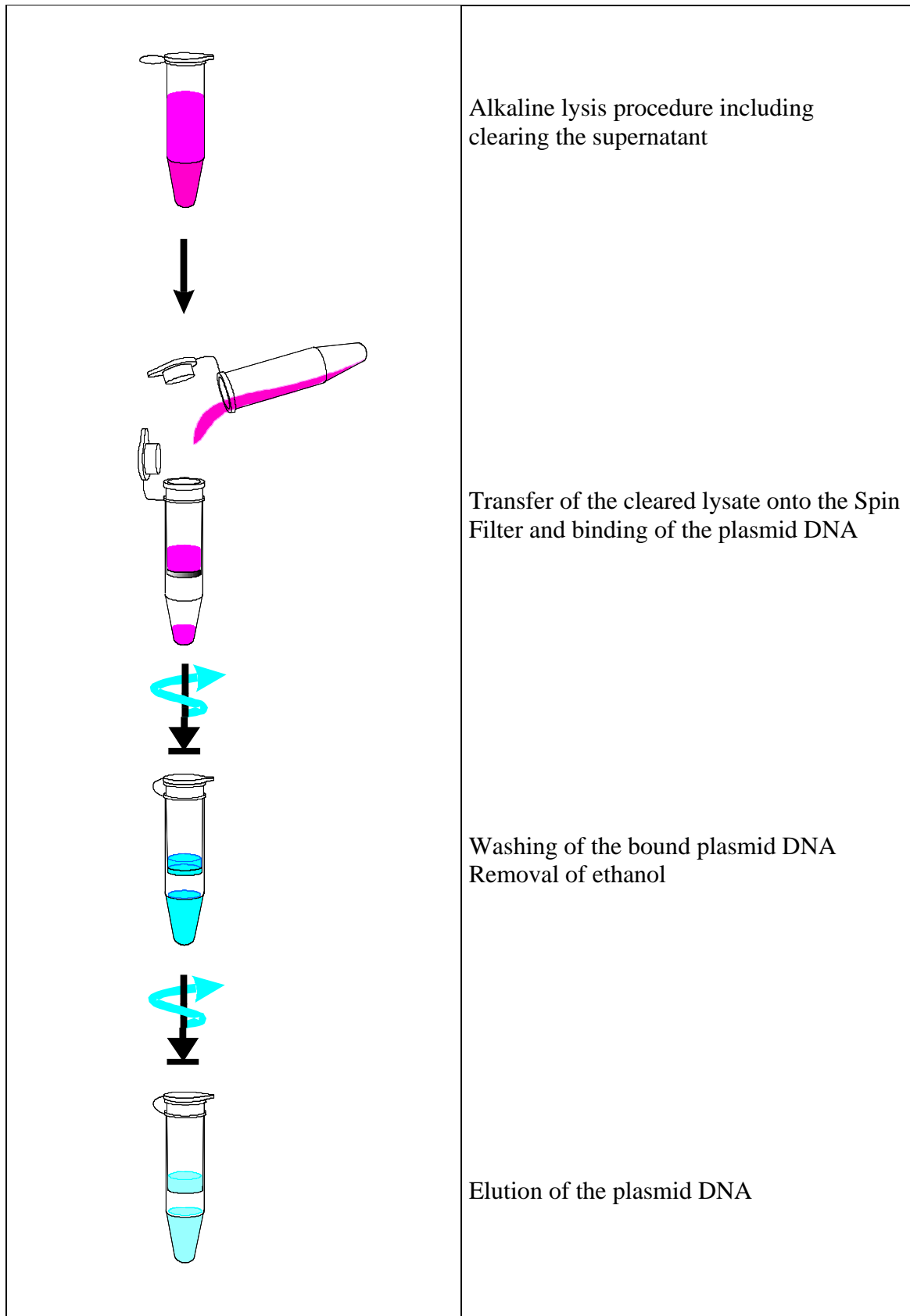
**For research use only!**

**Kit components (storage at room temperature)**

	10 plasmid DNA extractions *)	50 plasmid DNA extractions	250 plasmid DNA extractions	500 plasmid DNA extractions
<b>IBIAN Solution A</b>	2 x 2 ml	15 ml	70 ml	140 ml
<b>IBIAN Solution B</b>	2 x 2 ml	15 ml	70 ml	140 ml
<b>IBIAN Solution C</b>	2 x 2 ml	15 ml	70 ml	140 ml
<b>IBIAN Wash Solution</b>	10 ml (ready to use)	12 ml (final volume 60 ml)	1 x 40 ml (final volume 1 x 200 ml)	2 x 40 ml (final volume 2 x 200 ml)
<b>IBIAN Elution Solution</b>	2 ml	10 ml	30 ml	60 ml
<b>Spin Filter</b>	10	50	250	500
<b>2.0 ml Receiver Tubes</b>	10	50	250	500
<b>1.5 Receiver Tubes</b>	10	50	250	500
<b>Manual</b>	1	1	1	1
<b>Initial steps</b>	IBIAN Wash Solution is ready to use !	Add 48 ml 99.8% ethanol to the bottle IBIAN Wash Solution	Add 160 ml 99.8% ethanol to the bottle IBIAN Wash Solution	Add 160 ml 99.8% ethanol to each bottle IBIAN Wash Solution

\*) will be not provided in Spain

## General Scheme



**Protocol : Preparation of Plasmid DNA from 0.5 - 2.0 ml Bacteria cultures**

1. Transfer 0.5 ml up to 2 ml of the overnight *E. coli* culture into a 1.5 ml or 2.0 ml micro centrifuge tube. For larger volumes of starting material please contact our technical support.

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2. Centrifuge for 1 min at maximum speed to pellet the cells; remove the supernatant as completely as possible.

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3. Resuspend the cell pellet in **250 µl** of **IBIAN Solution A** completely by vortexing or by pipetting up and down.

**Note:** ♦ No cell pellet or clumps should be visible

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4. Add **250 µl** of **IBIAN Solution B**, close the tube and mix carefully by inverting the tube 5 times. **Do not perform the lysis step more than 5 min.**

**Important:** ♦ Do not vortex the tube to mix the suspension! This step is critical for the separation of bacterial chromosomal DNA from plasmid DNA. Mechanical stress by vortexing or extensive mixing leads to shearing of high-molecular weight chromosomal DNA. This sheared chromosomal DNA is not precipitated by NaOH/SDS and contaminates the plasmid DNA.

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5. Add **250 µl** of **IBIAN Solution C** and mix gently, but thoroughly, by shaking the tube 4-6 times. Centrifuge for 5 min at full speed 13,500 – 16,500 x g .

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6. During centrifugation time place the Spin Filter into the **2.0 ml Receiver Tubes**.

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7. Transfer (**decant !**) the clarified supernatant onto the **Spin Filter**. Incubate for 1 min. Centrifuge for 1 min at 10,000 x g.

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8. Discard the filtrate. Add **750 µl IBIAN Wash Solution**. Centrifuge for 1 min at 10,000 x g. Discard the filtrate.

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9. Centrifuge for 3 min at full speed 13,500 – 16,500 x g to complete removing the residual ethanol.

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10. Place the Spin Filter into a new **1.5 ml Receiver Tube** and add **50- 100 µl IBIAN Elution Solution**

directly onto the center of the Spin Filter surface. Incubate for 1 min at room temperature. Finally centrifuge at 10,000 x g for 1 min to elute the plasmid DNA.

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**Note:** ♦ To increase the final DNA yield we recommend to use a higher volume of Elution Buffer . A longer incubation time with Elution Buffer (up to 10 min) leads also to a slightly higher final yield. In order to increase the DNA concentration we recommend to elute in a smaller volume than 50 µl.

**Attention:** ♦ For in vitro transcription application, please elute the plasmid DNA with ddH<sub>2</sub>O. The IBIAN Elution Solution contains EDTA.

## Troubleshooting

Problem/probable cause	Comments and suggestions
<p><b>Low yield of plasmid DNA</b></p> <ul style="list-style-type: none"> <li>• Incorrect IBIAN Wash Solution</li> <li>• Poor elution of DNA</li> <li>• Conditions for bacterial cultures are not optimal</li> </ul>	<p>Prepare the IBIAN Wash Solution exactly as described in the manual Storage of IBIAN Wash Solution with firmly fixed cap</p> <p>Add the Elution Buffer directly on the center of the Spin Filter</p> <p>Changing of the conditions (media, growing time and other)</p>
<p><b>Additional band below the supercoiled plasmid DNA band</b></p> <ul style="list-style-type: none"> <li>• Denaturated supercoiled plasmid DNA</li> </ul>	<p>Incorrect incubation IBIAN Solution B. Increased incubation time with IBIAN Solution B can cause denaturation of supercoiled plasmid DNA</p>
<p><b>Contamination of the plasmid DNA with chromosomal DNA</b></p> <ul style="list-style-type: none"> <li>• The sample was mixed too vigorously during step 4</li> <li>• Bacteria overgrown</li> </ul>	<p>Follow exactly the protocol. Do not vortex at this step. Mix the samples only by inverting the tubes carefully</p> <p>Reduce time for growing the bacterial culture</p>
<p><b>Problems because of poor cleavage by restriction endonucleases or for other applications</b></p> <ul style="list-style-type: none"> <li>• Contamination of the final plasmid DNA with salt components</li> <li>• Contamination of the final DNA with ethanol</li> <li>• Contamination of the final DNA with carry over of filter material</li> </ul>	<p>Washing of the plasmid DNA bound on the Spin Filters as described</p> <p>Keep the given centrifugation time, extend it if necessary (test the smell)</p> <p>Centrifuge the eluted plasmid DNA at full speed for 1 minute and take a aliquot for the application directly from the surface.</p>