

# IBIAN<sup>®</sup> Fragment Clean-Up



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## Instruction for IBIAN<sup>®</sup> Fragment Clean-Up

**For ultra fast purification and concentration of PCR-fragments and of other enzymatic reaction mixtures as well as for the purification of DNA-fragments from agarose gels**

The **IBIAN<sup>®</sup> Fragment Clean-Up** provides a convenient tool for ultra fast and efficient direct purification of PCR\* products from 80 bp up to 30 kb from amplification reactions as well as for fast and efficient extraction and purification of DNA-fragments from agarose gels.

For PCR cleanup a special procedure is offered -no common used washing and drying steps are necessary. Finally, the DNA fragments will be eluted with low salt buffer or H<sub>2</sub>O.

No additional and common used washing and drying steps are necessary. Finally, the DNA fragments will be eluted with low salt buffer or H<sub>2</sub>O.

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



## IBIAN<sup>®</sup> Fragment Clean-Up

For purification of DNA-fragments from agarose gels, the DNA fragments from 80 bp up to 30 kb will be bound directly onto the surface of a spin filter column after gel solubilization. The DNA – fragments will be eluted in a low salt buffer after washing.

The extraction protocol as well as all buffers are optimized to provide high yield and purity of the recovered DNA-fragment. The “hands-on time“ necessary for the whole procedure is reduced to a minimum. The purification process will be ready in 5 - 20 minutes.

The purified DNA-fragments are ready to use in various downstream applications such as:

- Digestion with restriction enzymes
- Hybridization
- Labeling
- Cloning
- Sequencing
- *In vitro* Transcription

Name	Amount of starting material	Rate of recovery	Time for preparation
IBIAN <sup>®</sup> Fragment Clean-Up	up to 300 mg of agarose gel slices	60 % - 85 % depends on fragment length and kind of agarose gel (TAE or TBE)	less than 20 minutes
	up to 100 µl of amplification reaction volume	80 % - 95 % depends on fragment length	approx. 5 minutes

### Reagents and equipment to be supplied by user

- Ethanol (≥ 99.8 %)
- scalpel
- 1.5 ml or 2.0 ml reaction tubes
- Thermomixer or water bath for 50°C

\*) 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> Fragment Clean-Up** 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! The Buffer IBIAN Gel Solubilizer S contains a chaotropic substance and could be irritant.

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

## **Storage conditions**

The **IBIAN® Fragment Clean-Up** should be stored dry, at room temperature (14 – 25°C) and it is stable for at least 12 months under these conditions.

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® Fragment Clean-Up** for applications as described in the manual.

The components of each **IBIAN® Fragment Clean-Up** were tested by extraction of a DNA ladder from an agarose gel.

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® Fragment Clean-Up** 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**

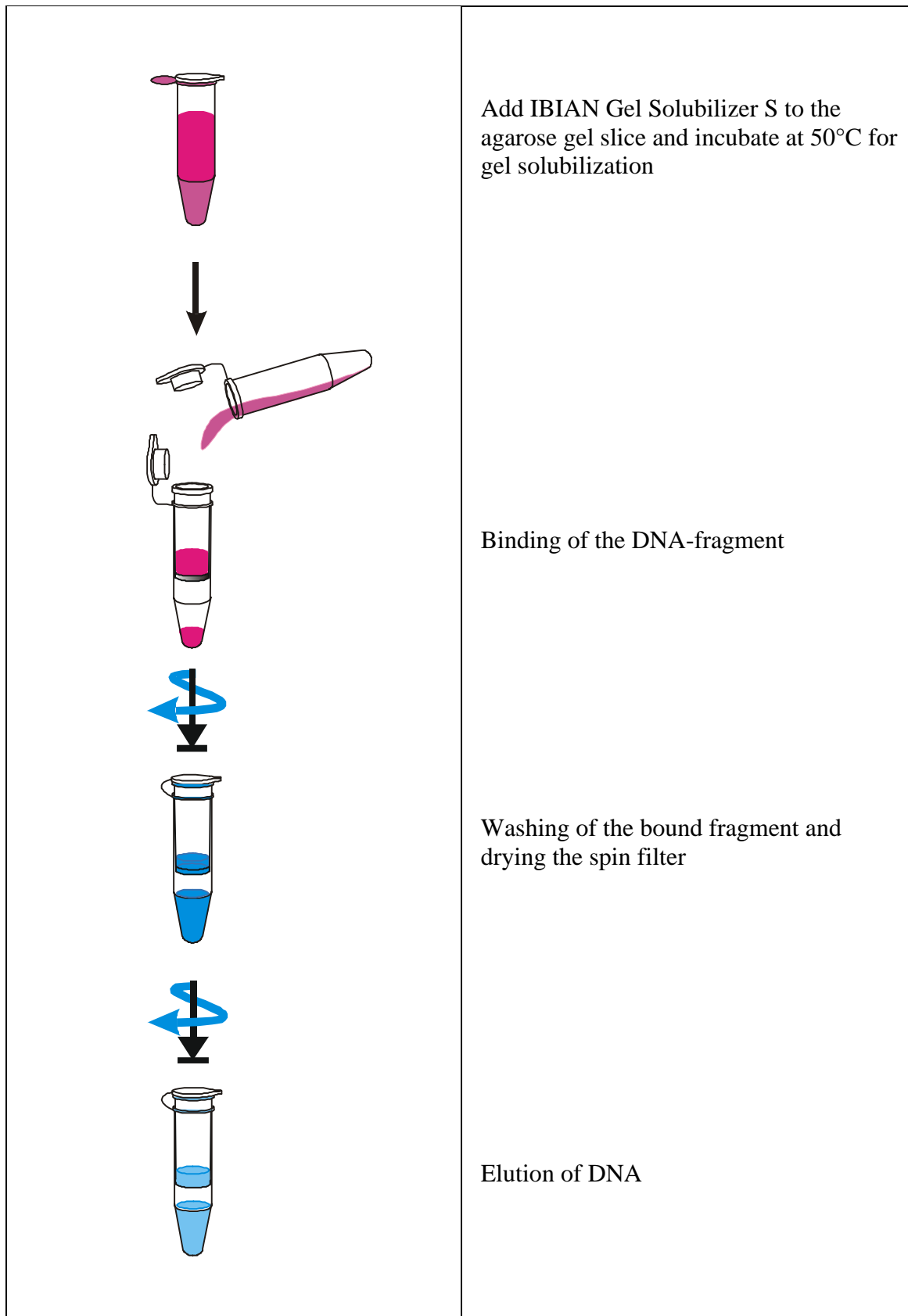
The user is responsible to validate the performance of the IBIAN kits for any particular use, since the performance characteristics of our kits have not been validated for any specific application. IBIAN kits may be used in clinical diagnostic laboratory systems after the laboratory has validated the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

**For research use only!**

### Kit components (storage at room temperature)

	10 purifications	50 purifications	250 purifications
<b>IBIAN Gel Solubilizer S</b>	12 ml	60 ml	2 x 140 ml
<b>IBIAN Binding Buffer</b>	6 ml	30 ml	140 ml
<b>IBIAN Binding Enhancer</b>	6 ml	30 ml	1 x 150 ml
<b>IBIAN Wash Buffer</b>	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</b>	2 ml	2 x 2 ml	15 ml
<b>Spin Filter</b>	10	50	250
<b>2.0 ml Receiver Tubes</b>	10	50	250
<b>1.5 ml Receiver Tubes</b>	10	50	250
<b>Manual</b>	1	1	1
<b>Initial steps</b>		<ul style="list-style-type: none"><li>• add 42 ml 96-100% ethanol to the bottle IBIAN Wash Buffer</li></ul>	<ul style="list-style-type: none"><li>• add 105 ml 96-100% ethanol to each bottle IBIAN Wash Buffer</li></ul>

**General Scheme of purification of DNA-fragments from agarose gels.**



## **Protocol : extraction of a DNA-fragment from an agarose gel slice**

**Important:** ♦ TBE-gels contain more potentially inhibitors for down stream application than TAE-gels. So we recommend the use of TAE-gels for critical downstream application!

**Important:** ♦ Before starting with the purification procedure please place a Spin Filter into a 2.0 ml Receiver Tube !

1. Excise the DNA-fragment from the agarose gel with a sharp scalpel. Minimize the agarose gel slice. Check the weight.

**For gel slices up to 150 mg add 500 µl IBIAN Gel Solubilizer S.**

**For gel slices > 150 mg add 1 ml of IBIAN Gel Solubilizer S.**

Do not use more than 300 mg gel slice for one Spin Filter.

Transfer the gel slice into a 1.5 or 2.0 reaction tube.

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2. Incubate at 50°C for 10 minutes until the agarose gel slice is completely dissolved. Incubation under continuous shaking (e.g. Eppendorf Thermomixer) is very helpful.

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3. Add 250 µl IBIAN Binding Enhancer to a 500 µl reaction volume or 500 µl IBIAN Binding Enhancer to a 1 ml reaction volume and mix the suspension by pipetting some times or by vortexing.

Load appr. 800 µl of the sample onto the Spin Filter. Centrifuge at 10,000 – 12,000 rpm for 1 minute. Discard the filtrate. For reaction volumes > 800 µl reload the residual volume onto the Spin Filter and repeat the centrifugation step.

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4. Add 500 µl IBIAN Wash Buffer to the Spin Filter and centrifuge for 30 sec at 12,000 rpm. Discard the filtrate. Repeat the washing step once again.

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5. Discard the filtrate. Remove the residual ethanol of the IBIAN Wash Buffer by centrifugation for 4 min at maximum speed (12,000 – 14,000 rpm).

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6. Transfer the Spin Filter into a new 1.5 ml Receiver Tube.

Add at least 20 µl IBIAN Elution Buffer directly onto the center of the Spin Filter.

Incubate at room temperature for 5 minutes. Centrifuge for 1 minute at 12,000 rpm.

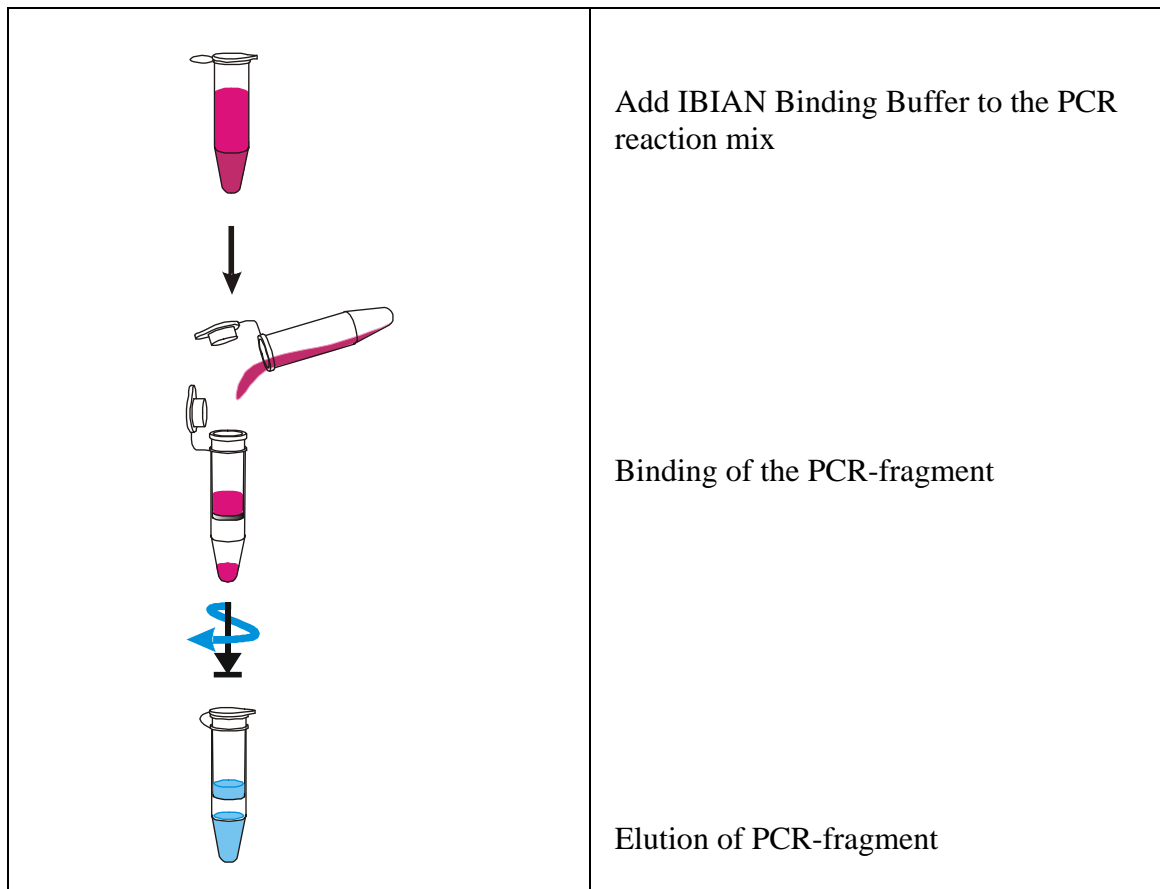
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**Note:** ♦ To increase the final DNA yield we recommend to use a higher volume of IBIAN Elution Buffer.

Please take into account that an increasing volume of IBIAN Elution Buffer reduces the final concentration of the purified DNA.

An extended incubation time with IBIAN Elution Buffer (up to 10 minutes) leads also to a slightly higher final yield.

## General Scheme of purification of PCR\* products



## **Protocol: Purification and concentration of PCR-products from PCR reactions**

**Note:** ♦ Before starting with the purification procedure please place a Spin Filter into a 2.0 ml Receiver Tube !

### **1. Binding of the PCR-fragments**

#### **A. For PCR-mixtures up to 50 µl**

Add **250 µl IBIAN Binding Buffer** to the PCR sample and mix very well by pipetting or vortexing.

Transfer the sample completely onto a Spin Filter and centrifugate for 3 min at 12.000 rpm.

#### **B. For PCR-mixture > 50 µl up to 100 µl**

Add **500 µl IBIAN Binding Buffer** to the PCR sample and mix very well by pipetting or vortexing. Transfer the sample completely onto a Spin Filter and centrifugate for 1 min at 12.000 rpm. Remove the filtrate and centrifuge again for 2 minutes.

### **2. Elution of the PCR-fragments**

Place the Spin Filter into a new 1.5 ml Receiver Tube.

Add at least 10 µl IBIAN Elution Buffer (or ddH<sub>2</sub>O) directly onto the center of the Spin Filter. Incubate for 1 minute at room temperature. Centrifugation for 1 minute at 10.000 rpm.

**Important Notes:**

1. If the PCR-mixture contains mineral oil, we recommend the addition of 500 µl of IBIAN Binding Buffer\* independent of the starting volume. It is also possible to wash the bound PCR-fragment once with 500 µl of IBIAN Binding Buffer.

2. To increase the final DNA yield we recommend an extended incubation time with IBIAN Elution Buffer (up to 5 minutes), which will lead to a slightly higher final yield.

3. For concentration of PCR-fragments it is possible to elute with lower volume of IBIAN Elution Buffer, than the volume of the starting PCR-mixture. The minimum volume is 10 µl.

You can order additional IBIAN Binding Buffer for this application by phone (+34-976 141 693) or by mail:

[info@ibiantech.com](mailto:info@ibiantech.com)

**Troubleshooting**

<b>Problem/ probable cause</b>	<b>Comments and suggestions</b>
<b>Low recovery</b> Poor elution of DNA	Add the elution buffer directly onto the center of the Spin Filter (even if a small elution volume is used). Apply the correct centrifugation steps
Problems with mineral oil	Take a higher volume of IBIAN Binding Buffer Wash once with IBIAN Binding Buffer
Incorrect IBIAN Wash Buffer or no ethanol added	Prepare the IBIAN Wash Buffer exactly as described in the manual. Storage of IBIAN Wash Buffer with firmly fixed cap.
TBE buffered gels are used	The binding of DNA fragments under TBE buffer condition is slightly reduced. For smaller fragments than 500 bp please use TAE agarose gels.
Ineffective solubilization of the agarose gel slice	The gel slice must be completely dissolved.
No IBIAN Binding Enhancer added	Add the amount of IBIAN Binding Enhancer needs to the solubilized suspension.
<b>Problems with down stream application, e.g. ligation</b>	
• Contamination with salt components	Washing of the Spin Filters as described in the manual. Prolong the incubation time with IBIAN Wash Buffer to 5 minutes before centrifugation.
• Contamination with agarose traces	Wash the Spin Filter one time with IBIAN Gel Solubilizer S.
• Contamination of the final DNA with ethanol	Keep the given centrifugation time, extend it if necessary (test the smell).