

IBIAN[®] dye Cleanup



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Instruction for IBIAN[®] dye Cleanup

For ultra fast purification and concentration of PCR-fragments

The **IBIAN[®] dye Cleanup** provides a convenient tool for ultra fast and efficient direct purification of PCR* products from 80 bp up to 30 kb from amplification reactions and for reliable removal of DyeDeoxy[™] terminators from DNA-cycle sequencing reactions. The DNA-fragments will be bound directly onto the surface of a spin filter column based on new buffer composition.

No additional and common used washing and drying steps are necessary. Finally, the DNA fragments will be eluted with low salt buffer or H₂O

*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[®] dye Cleanup

The new-patented technology for binding of DNA fragments onto the spin filter surface does not need any hazardous chaotropic buffer.

The isolation protocol as well as all buffers is optimized to provide high yield and purity of the recovered DNA fragment and for effective removal of free and labelled dNTP's from fluorescent reaction mixtures. The "hands-on time" necessary for the whole procedure is reduced to a minimum. The purification process will be ready in approximately 4 minutes.

The purified DNA fragments are ready to use in various downstream application 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[®] dye Cleanup	up to 100 µl of amplification reaction volume	80 % - 95 % depends on fragment length	approximately 4 minutes

*) 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[®] dye Cleanup** cannot be construed as an authorization or implicit license 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, flush eyes or skin with a large amount of water immediately.

Storage conditions

The **IBIAN[®] dye Cleanup** should be stored dry, at room temperature (14 – 25°C) and 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 **IBIAN[®] dye Cleanup** for applications as described in the manual. The components of each **IBIAN[®] dye Cleanup** were tested by purification of PCR fragments from different length.

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 **IBIAN[®] dye Cleanup** 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.

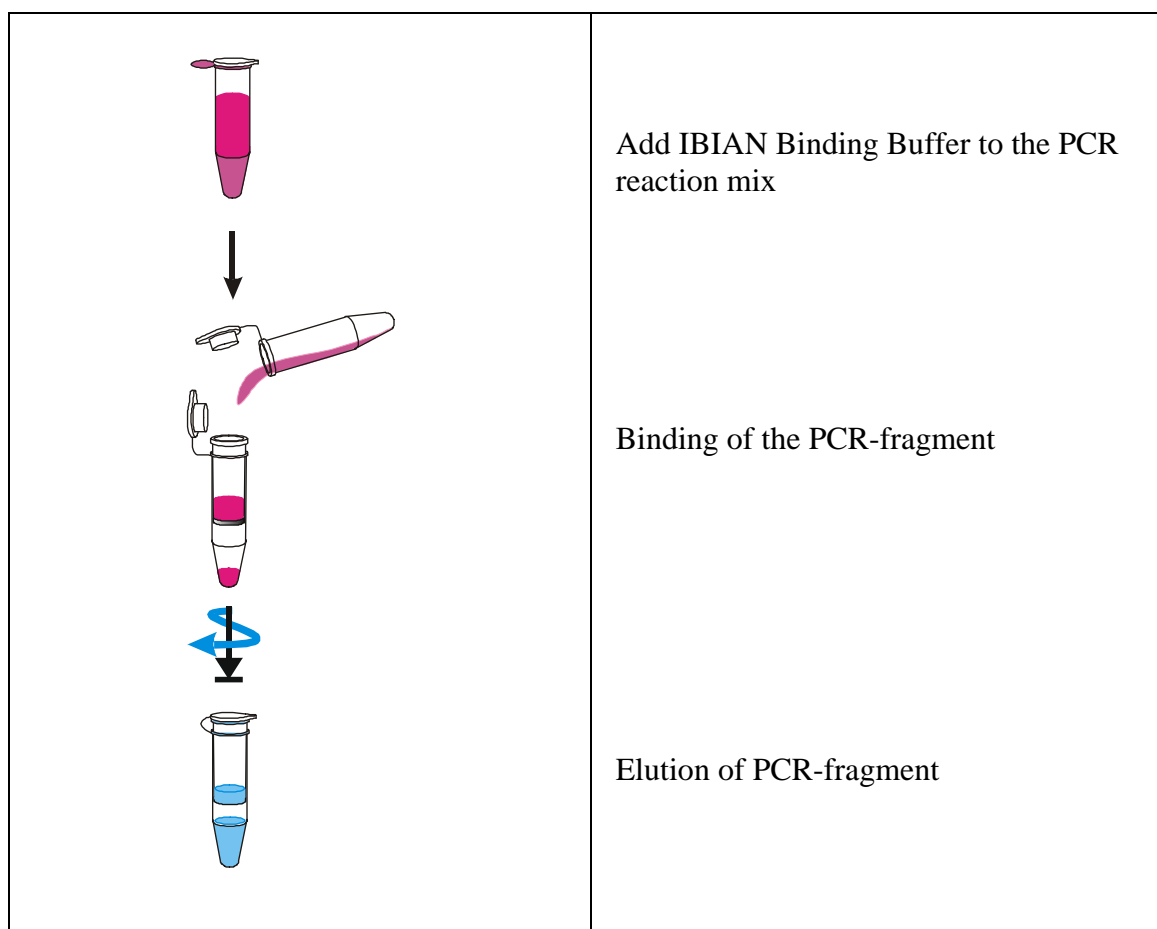
All products sold by IBIAN are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately.

For research use only!

Kit components (storage at room temperature)

	10 purifications	50 purifications	250 purifications	500 purifications
IBIAN Binding Buffer 1	6 ml	30 ml	140 ml	2 x 140 ml
IBIAN Binding Buffer 2	6 ml	30 ml	140 ml	2 x 140 ml
IBIAN Elution Buffer	2 ml	3 x 2 ml	30 ml	60 ml
Spin Filter	10	50	250	500
2.0 ml Receiver-Tubes	10	50	250	500
1.5 ml Receiver-Tubes	10	50	250	500
Manual	1	1	1	1

General Scheme



Protocol 1: Purification and concentration of PCR-products from PCR reactions for removal of dNTP's and Primers

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 1** to the PCR sample and mix very well by pipetting or vortexing.

Transfer the sample completely onto a Spin Filter and centrifuge for 4 min at maximum speed.

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

Add **500 µl IBIAN Binding Buffer 1** to the PCR sample and mix very well by pipetting or vortexing. Transfer the sample completely onto a Spin Filter and centrifuge 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 ddH₂O) directly onto the center of the Spin Filter. Incubate for 1 minute at room temperature. Centrifuge 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 1 independent of the starting volume. It is also possible to wash the bound PCR-fragment once with 500 µl of IBIAN Binding Buffer 1.
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.

Protocol 2: Purification and concentration of PCR-products *over 100 bp* with contaminations of primer dimers or shorter fragments*

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

Add 10 volumes of IBIAN Binding Buffer 2 to 1 volume of the PCR reaction (max. 50 µl) and mix very well by pipetting or vortexing.

Transfer the sample completely onto a Spin Filter and centrifuge for 4 min at maximum speed.

2. Elution of the PCR-fragments

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

Add at least the IBIAN Elution Buffer (or ddH₂O) in **the same volume** as the original PCR reaction (**min. 10 µl**) directly onto the center of the Spin Filter.

Incubate for 1 minute at room temperature. Centrifuge 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 2 independent of the starting volume. It is also possible to wash the bound PCR-fragment once with 500 µl of IBIAN Binding Buffer 2.
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.
4. At very short PCR-fragments you may get reduced yield so we recommend at least 100 bp in length. Comparing a Primer Dimer of 70 bp and a PCR-Product of 80 bp there is no big difference. For the purification of short products without contaminations of Dimers we recommend the use of protocol 1.

*Especially in DNA-Sequencing and PCR product cloning Primer Dimers or contamination with short DNA fragments creates problems. T-Vector Cloning kits preferably accept shorter fragments, and the contamination leads to a high cloning background of false clones. In sequencing false fragments like Primer Dimers may lead to high

background in the starting region of the sequence.

Protocol 3: Removal of DyeDeoxy™ terminators from DNA cycle sequencing reactions of PCR-products and Plasmids after use ABI Prism™ terminator Kits

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

1. Binding of the (fluorescent) labelled DNA

Add **500 µl IBIAN Binding Buffer 1** to the completed cycle sequencing reaction and mix very well by pipetting or vortexing. Transfer the sample completely onto a Spin Filter and centrifuge for 4 min at at maximum speed.

2. Elution of the (fluorescent) labelled DNA

Place the Spin Filter into a new 1.5 ml Receiver Tube.
Add at least 5 µl IBIAN Elution Buffer (or ddH₂O) directly onto the center of the Spin Filter.
Incubate for 1 minute at room temperature. Centrifuge for 1 minute at 10.000 rpm.
Discard the Spin Filter and proceed with the ABI sample loading.

Troubleshooting

Problem/ probable cause	Comments and suggestions
Low recovery Poor elution of DNA Problems with mineral oil	 Add the IBIAN Elution Buffer directly onto the center of the Spin Filter (even if a small elution volume is used). Apply the correct centrifugation steps Take a higher volume of IBIAN Binding Buffer Wash once with IBIAN Binding Buffer