

IBIAN[®] DNA Stool Kit



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IBIAN[®] DNA Stool Kit

For DNA extractions from 200-400 mg stool

Name: IBIAN[®] DNA Stool Kit

Amount of starting material: 200-400 mg of stool sample

Yield: up to 65 µg (depends on starting material)

Time for preparation: app. 45 minutes

Ratio: A₂₆₀ / A₂₈₀ (1.6 – 1.8)

The **IBIAN[®] DNA Stool Kit** provides a rapid and efficient way to purify high quality DNA from up to 200 – 400 mg of fresh or frozen stool sample. The **IBIAN[®] DNA Stool Kit** combines the lysis of starting material with the very efficient binding of DNA onto a Spin Filter surface based on a new and sui generis patented technology without chaotropic substances.

The fast **IBIAN[®] DNA Stool Kit** procedure yields high-quality DNA while efficiently removing impurities and enzyme inhibitors in a minimum time. The “hands-on time“ required for the whole process is reduced to minimum.

The purified DNA is ready to use in most downstream applications like PCR*, RLFP, Hybridization and other for:

- Genetic typing
- Pathogen typing
- Mutational analysis

Reagents and equipment to be supplied by user

- Microcentrifuge
- Thermomixer (for 70°C/95°C)
- 1.5 ml tubes;
- 99.8% ethanol

*) 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[®] DNA Stool Kit** cannot be construed as an authorization or implicit licence to practice PCR under any patents held by Hoffmann-LaRoche Inc.

Storage conditions

The **IBIAN[®] DNA Stool Kit** should be stored dry, at room temperature (14 – 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[®] DNA Stool Kit** for applications as described in the manual. The components of each **IBIAN[®] DNA Stool Kit** were tested isolation of genomic DNA from 200 mg of a frozen stool sample, including gel electrophoresis and spectrophotometry.

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® DNA Stool Kit** or other IBIAN products, please do not hesitate to contact us. For technical support or further information in Spain please dial +34-976 90 16 45.

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.

Safety precautions

Take the appropriate safety measures for working in laboratories, especially for working with biological material. The Wash Buffer I contains a chaotropic component.

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

IBIAN Binding Buffer : 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)

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	3 x 2 ml	15 ml	120 ml	2 x 160 ml
IBIAN Binding Buffer	2 ml	3 x 2 ml	30 ml	120 ml
Proteinase K	for 250 µl working solution	for 250 µl working solution	for 1.5 ml working solution	for 5 x 1.5 ml working solution
IBIAN Wash Buffer I	15 ml (ready to use)	15 ml (ready to use)	30 ml (final volume 60 ml)	80 ml (final volume 160 ml)
IBIAN Wash Buffer II	15 ml (ready to use)	15 ml (ready to use)	18 ml (final volume 60 ml)	2 x 45 ml (final vol. 2 x 150 ml)
IBIAN Elution Buffer	2 ml	2 x 2 ml	30 ml	60 ml
Safe-Lock-Tubes 2.0 ml	3	10	50	250

IBIAN Inhibitor Remove	3	10	50	250
Receiver Tubes 2.0 ml	3	10	50	250
Receiver Tubes 1.5 ml	3	10	50	250
Spin Filter	3	10	50	250
Manual	1	1	1	1
Initial steps	<ul style="list-style-type: none"> • Add 250 µl dd H₂O to the tube Proteinase K, mix thoroughly and store the tube at -20°C! • Incubate the needed amount of IBIAN Elution Buffer at 70°C in a Thermomixer 	<ul style="list-style-type: none"> • Add 250 µl dd H₂O to the tube Proteinase K, mix thoroughly and store the tube at -20°C! • Incubate the needed amount of IBIAN Elution Buffer at 70°C in a Thermomixer 	<ul style="list-style-type: none"> • Add 30 ml of 96-100% Ethanol to the bottle IBIAN Wash Buffer I • Add 42 ml of 96-100% Ethanol to the bottle IBIAN Wash Buffer II, mix thoroughly and store with tightly closed cap. • Add 1,5 ml dd H₂O to the tube Proteinase K, mix thoroughly and store the tube at -20°C! • Incubate the needed amount of IBIAN Elution Buffer at 70°C in a Thermomixer 	<ul style="list-style-type: none"> • Add 80 ml of 96-100% Ethanol to the bottle IBIAN Wash Buffer I • Add 105 ml of 96-100% Ethanol to each bottle IBIAN Wash Buffer II, mix thoroughly and store with tightly closed cap. • Add 1,5 ml dd H₂O to each tube Proteinase K, mix thoroughly and store the tube at -20°C! • Incubate the needed amount of IBIAN Elution Buffer at 70°C in a Thermomixer

Protocol : DNA isolation from stool sample

Important: Please note, that the extracted DNA for stool sample is by the majority from bacterial origin !

Prewarm the IBIAN Elution Buffer to 70°C (e.g. transfer the needed volume into a tube and place it at the appropriate temperature into a thermomixer)

1. Sample Homogenization and Prelysis

Weigh 200 - 400 mg of stool sample (fresh or frozen) into a 2.0 ml Safe-Lock-Tube.

Important: If the sample is liquid, pipet 200 - 400 µl into the 2.0 ml Safe-Lock-Tube. Cut the end of the pipet tip to make pipetting easier.

Add 1.2 ml IBIAN Lysis Buffer to each stool sample .Vortex vigorously for 1 min Incubate the sample for 10 min at 95°C in a thermomixer under continuously shaking at 900 rpm. Centrifuge the sample at 14,000 rpm for 1 min to pellet solid stool particles.

Important: The incubation step at 95°C will lead to maximize the amount of bacterial DNA, because of a very efficient destruction of the cell wall of e.g. gram+ bacteria. If you want enrich human DNA incubate the sample in the

IBIAN Lysis Buffer for 10 min at RT instead of at 95° C !

2. First Sample Cleanup

Transfer the supernatant into a IBIAN Inhibitor Remove -Tube and vortex vigorously for 15 sec. Incubate the suspension for 1 min at room temperature. Centrifuge the sample at 14,000 rpm for 3 min.

3. Second Sample Cleanup

Transfer the supernatant completely into a new 1.5 ml centrifuge tube; discard the pellet. Centrifuge the sample at 14,000 rpm for 3 min.

4. Proteinase K Digestion

Transfer 25 µl Proteinase K into a new 1.5 ml centrifuge tube and pipett 400 µl of the supernatant from step 3 to the 1.5 ml centrifuge tube containing Proteinase K, mix shortly by vortexing and incubate the sample for 10 min at 70°C in a thermomixer under continuously shaking at 900 rpm.

5. Binding of the DNA onto the Spin Filter

Add 400 µl of IBIAN Binding Buffer to the lysate and mix shortly by vortexing or by pipetting up and down several times.

Place the Spin Filter in a 2.0 ml Receiver Tube and transfer the lysate onto the Spin Filter.

Incubate for 1 min at room temperature and centrifuge at 12,000 rpm for 1 min.

Discard the filtrate.

6. First Washing of the Spin Filter

Add 500 µl of IBIAN Wash Buffer I to the Spin Filter and centrifuge at 12,000 rpm for 1 min.

Discard the filtrate.

7. Second Washing of the Spin Filter

Add 800 µl of IBIAN Wash Buffer II to the Spin Filter and centrifuge at 12,000 rpm for 1 min.

Discard the filtrate.

8. Removing of Ethanol

To remove the ethanol centrifuge at 12,000 – 14,000 rpm for 3 min.

9. Elution of the bound DNA

Place the Spin Filter into a new 1.5 ml Receiver Tube and add 100 - 200 µl prewarmed (70°C) IBIAN Elution Buffer. Incubate for 3 min. Centrifuge at 8,000 rpm for 1 min to elute the DNA. Finally discard the Spin Filter.

Note: ♦ For long-term storage, we recommended to keep the eluted DNA at –20°C.

Troubleshooting

Problem/probable cause	Comments and suggestions
Clogged Spin Filter <ul style="list-style-type: none"> Insufficient lysis and/or too much starting material 	Increase lysis time. Increase centrifugation speed or time. Reduce amount of starting material.
Low amount of extracted DNA <ul style="list-style-type: none"> Insufficient lysis Incomplete elution Insufficient mixing with Binding Buffer P 	Increase lysis time. Reduce amount of starting material. Overloading of Spin Filter reduces yield! Prolong the incubation time with Elution Buffer to 5-10 min or repeat elution step once again. Take higher volume of IBIAN Elution Buffer. Mix sample with IBIAN Binding Buffer by pipetting or by vortexing prior to transfer the sample onto the Spin Filter.
Spin Filter Surface turns yellow <ul style="list-style-type: none"> Incomplete lysis Insufficient washing 	See above Wash again with IBIAN Wash Buffer II
Low concentration of extracted DNA <ul style="list-style-type: none"> Too much IBIAN Elution Buffer 	Elute the DNA with lower volume of IBIAN Elution Buffer.
Degraded or sheared DNA <ul style="list-style-type: none"> incorrect storage of starting material Old material 	Ensure that the starting material is fresh or stored under appropriate condition (for long time storage at – 20°C)! Avoid thawing and freezing of the material. Old material often contains degraded DNA.
RNA contaminations of extracted DNA.	RNase A digestion