

Instructions for Use

Life Science Kits & Assays

A glass pipette tip is shown splashing water. Inside the pipette, a blue DNA double helix is visible. The background is a gradient of blue and black.

innuPREP PCRpure Kit

Order No.:

845-KS-5010010 10 reactions
845-KS-5010050 50 reactions
845-KS-5010250 250 reactions

Publication No.: HB_KS-5010_e_211020

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It needs not necessarily agree with future versions. Subject to change!

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Contents

1	Introduction.....	2
1.1	Intended use.....	2
1.2	Notes on the use of this manual and the kit.....	3
2	Safety precautions.....	4
3	Storage conditions	5
4	Functional testing and technical assistance.....	6
5	Product use and warranty.....	6
6	Kit components	7
7	Product specifications	8
8	Protocol: Purification and concentration of PCR products from PCR reactions up to 50 µl	9
9	Protocol: Purification of sequencing reaction mixes	11
10	Troubleshooting.....	12

1 Introduction

1.1 Intended use

The innuPREP PCRpure Kit has been designed for the purification and concentration of PCR products from PCR reactions up to 50 µl and sequencing reaction mixtures. The kit uses Spin Filter technique in combination with a new patented chemistry which requires no washing steps resulting in a very fast and reliable purification of DNA fragments.

The kit is intended for use by professional users.












CONSULT INSTRUCTION FOR USE

This package insert must be read carefully before use. Package insert instructions must be followed accordingly. Reliability of results cannot be guaranteed if there are any deviations from the instructions in this package insert.

1.2 Notes on the use of this manual and the kit

For easy reference and orientation, the manual and labels use the following warning and information symbols as well as the shown methodology:

Symbol	Information
	REF Catalogue number.
	Content Contains sufficient reagents for <N> tests.
	Storage conditions Store at room temperature or shown conditions respectively.
	Consult instructions for use This information must be observed to avoid improper use of the kit and the kit components.
	Expiry date
	Lot number The number of the kit charge.
	Manufactured by Contact information of manufacturer.
	For single use only Do not use components for a second time.
	Note / Attention Observe the notes marked in this way to ensure correct function of the device and to avoid operating errors for obtaining correct results.

The following systematic approach is introduced in the manual:

- The chapters and figures are numbered consecutively.
- A cross reference is indicated with an arrow (e.g. → “Notes on the use of this manual” p. 3).
- Work steps are numbered.

2 Safety precautions

NOTE

Read through this chapter carefully before to guarantee your own safety and a trouble-free operation.

Follow all the safety instructions explained in the manual, as well as all messages and information, which are shown.

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.



FOR SINGLE USE ONLY!

This kit is made for single use only!

ATTENTION!

Don't eat or drink components of the kit!

The kit shall only be handled by educated personnel in a laboratory environment!

If the buffer bottles are damaged or leaking, wear gloves and protective goggles when discarding the bottles in order to avoid any injuries. This kit could be used with potential infectious samples. Therefore, all liquid waste must be considered as potentially infectious and must be handled and discarded according to local safety regulation.

Please observe the federal, state and local safety and environmental regulations. Follow the usual precautions for applications using extracted nucleic acids. All materials and reagents used for DNA or RNA isolation should be free of DNases or RNases.

ATTENTION!

Do not add bleach or acidic components to the waste after sample preparation!

NOTE

Emergency medical information in English and German can be obtained 24 hours a day from:

Poison Information Center, Freiburg / Germany

Phone: +49 (0)761 19 240.

For more information on GHS classification and the safety data sheet (SDS) please contact sds.innu@ist-ag.com.

3 Storage conditions

The kit is shipped at ambient temperature.

The innuPREP PCRpure Kit should be stored dry at room temperature (15 °C to 30 °C). When stored at room temperature, the kit is stable until the expiration date printed on the label on the kit box.

If there are any precipitates within the provided solutions solve these precipitates by careful warming. Before every use make sure that all components have room temperature.

For further information see chapter "Kit components" (→ p. 7).

4 Functional testing and technical assistance

The IST Innuscreen GmbH guarantees the correct function of the kit for applications as described in the manual. This product has been produced and tested in an ISO 13485 certified facility.

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 the innuPREP PCRpure Kit or other IST Innuscreen GmbH products, please do not hesitate to contact us.

For technical support or further information in Germany please dial +49 30 9489 3380. For other countries please contact your local distributor.

5 Product use and warranty




The kit is not designed for the usage of other starting materials or other amounts of starting materials than those, referred to in the manual (→ "Intended use" p. 2) (→ "Product specifications" p. 8). Since the performance characteristics of IST Innuscreen GmbH kits have just been validated for the application described above, the user is responsible for the validation of the performance of IST Innuscreen GmbH kits using other protocols than those described below. IST Innuscreen GmbH 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 equivalent regulations required in other countries.

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

NOTE

The kit is for research use only!

6 Kit components

	 10	 50	 250
REF	845-KS-5010010	845-KS-5010050	845-KS-5010250
Binding Buffer	6 ml	30 ml	140 ml
Elution Buffer	2 ml	2 x 2 ml	15 ml
Spin Filter	10	50	5 x 50
Receiver Tubes (2.0 ml)	10	50	5 x 50
Elution Tubes (1.5 ml)	10	50	5 x 50
Manual	1	1	1

7 Product specifications

1. Starting material:
 - PCR reaction mixtures (up to 50 μ l)
 - Sequencing reaction mixtures

2. Time for extraction:
 - 3 minutes
 - Based on a new two-step procedure

3. Fragment length:
 - 60 bp–30 kbp

4. Binding capacity:
 - > 20 μ g DNA

5. Rate of recovery:
 - 60–90 %
 - Depending on the length of PCR fragments

8 Protocol: Purification and concentration of PCR products from PCR reactions up to 50 µl

NOTE

Before starting with the purification procedure place a Spin Filter into a 2.0 ml Receiver Tube.

A Binding of the PCR fragments

1. Add 500 µl Binding Buffer to the Spin Filter.
2. Add up to 50 µl of your PCR reaction mixture to the Spin Filter which is already pre-filled with the Binding Buffer.
3. Mix Binding Buffer and PCR reaction mixture by pipetting three times up and down. Don't destroy the filter membrane!

Alternatively

Mix 500 µl Binding Buffer with up to 50 µl of the PCR reaction mixture very well by pipetting or vortexing outside the Spin Filter in a separate reaction tube.

After this transfer the mixed sample completely onto the Spin Filter.

4. Centrifuge for 3 minutes at 11,000 x g (~11,000 rpm). Discard the Receiver Tube.
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NOTE

Avoid any contact of the Spin Filter with the flow through.

For maximum purity, discard the flow through after a first centrifugation step for 1 minute at 11,000 x g (~11,000 rpm) and place the Spin Filter back into the Receiver Tube and centrifuge for another 2 minutes at 11,000 x g (~11,000 rpm).

B Elution of the PCR fragments

1. Place the Spin Filter into an Elution Tube.
2. Pipette at least **10 μ l Elution Buffer** (minimum, see note) or RNase-free water directly onto the center of the Spin Filter.
3. Incubate for 1 minute at room temperature.
4. Centrifuge for 1 minute at 11,000 x g (~11,000 rpm). The Elution Tube now contains the purified PCR fragments.

NOTE

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

For concentration of PCR fragments, it is possible to perform the elution with a lower volume of **Elution Buffer** than the volume of the starting PCR mixture. The minimum volume is 10 μ l.

9 Protocol: Purification of sequencing reaction mixes

NOTE

Before starting with the purification procedure place a Spin Filter into a 2.0 ml Receiver Tube.

A Binding of the DNA fragments

1. Mix **300 µl Binding Buffer** with the **sequencing reaction sample** very well by pipetting or vortexing.
2. Transfer the sample completely onto a Spin Filter and centrifuge for 3 minutes at 11,000 x g (~11,000 rpm).
3. Discard the Receiver Tube.

B Elution of the DNA fragments

1. Place the Spin Filter into an Elution Tube.
2. Pipette at least **10 µl Elution Buffer** (minimum, see note) or RNase-free water directly onto the center of the Spin Filter.
3. Incubate for 1 minute at room temperature.
4. Centrifuge for 1 minute at 11,000 x g (~11,000 rpm). The Elution Tube now contains the purified PCR fragments.

NOTE

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

10 Troubleshooting

Problem / probable cause	Comments and suggestions
Low recovery / poor quality	
Poor elution of DNA	Add the Elution Buffer directly onto the center of the Spin Filter (even if a small elution volume is used).
Poor quality of DNA	<p>After binding of DNA an additional washing step with 80 % ethanol is recommended. Only use pure/non denatured ethanol.</p> <p>Add 600 µl of 80 % ethanol to the Spin Filter and centrifuge for 1 minute at 11,000 x g. Discard the flow through.</p> <p>For removing the ethanol centrifuge for 2 minutes at 11,000 x g.</p> <p>Continue with the elution step as described.</p>
Problems with mineral oil	Increase volume of Binding Buffer .

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