Instructions for Use
Life Science Kits & Assays

innuPREP PCRpure Lite Kit

Innuscreen GmbH

Order No.:
845-KS-5011100 100 reactions

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1 Introduction

1.1 Intended use

The **innuPREP PCRpure Lite Kit** has been designed as a extremely fast, simple and highly efficient method for purifying amplification products directly from PCR reaction mixtures and/or for concentrating PCR products.

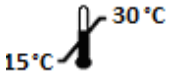
The purification procedure is based on a two-step method and takes only approx. 5 minutes to complete. The need for previously standard wash steps is eliminated, thereby reducing the overall process to binding and elution.

The process makes it possible to recover amplification products ranging in size from > 60 bp to 30 kb with recovery rates of 75 % to 95 % depending on the length of the amplification product. Also, elution can be performed with a very small volume of just 10 µl, which eliminates the need for specialized “mini-elute” Spin Filter columns.

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 and the kit” p. 3).
- Working 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 Lite 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.

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 contact info.innu@ist-ag.com. 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 (→ “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

6.1 Components included in the kit

	Σ 100
REF	845-KS-5011100
Binding Buffer	60 ml
Spin Filter	2 x 50
Receiver Tubes (2.0 ml)	2 x 50
Manual	1

6.2 Components not included in the kit

- Ultrapure Water/ddH_2O or Elution Buffer (10 mM Tris, pH 8.0 – 8.5)
- 1.5 ml reaction tubes

NOTE

Centrifugation steps should be carried out at room temperature.

7 Product specifications

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

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

3. Fragment length:
   - 60 bp–30 kbp

4. Binding capacity:
   - > 20 µg 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 located in a 2.0 ml Receiver Tube.
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 **2 minutes** at 11,000 x g (~11,000 rpm). Discard the Receiver Tube.

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 a 1.5 ml reaction tube (not included).
2. Pipette at least **10 µl ultrapure Water** (or 10 mM Tris, pH 8.0-8.5) directly onto the center of the Spin Filter.
3. Incubate for 1 minute at room temperature.
4. Centrifuge for 1 minute at 6,000 x g (~8,000 rpm). The 1.5 ml reaction tube contains the purified PCR fragments now.

NOTE

To increase the final DNA yield we recommend an extended incubation time with ultrapure Water or 10 mM Tris (pH 8.0-8.5) 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 ultrapure Water or 10 mM Tris (pH 8.0-8.5) than the volume of the starting PCR mixture. The minimum volume is 10 µl.

If the volume of the PCR reaction mix is higher than 50 µl, split the PCR mix and add to each part 500 µl Binding Buffer. Load both mixes one after another (successively) on the Spin Filter. Centrifuge the first part for 1 minute and discard the filtrate. Centrifuge the second part of the mix for 2 minutes. Follow now the elution step as described above.

9 Troubleshooting

Problem / probable cause	Comments and suggestions
Low recovery / poor quality	
Poor elution of DNA	Add the ultrapure Water or 10 mM Tris (pH 8.0-8.5) directly onto the center of the Spin Filter (even if a small elution volume is used). Apply the correct centrifugation steps
Poor quality of DNA	After binding of DNA an additional washing step with **80 % ethanol** is recommended. Only use pure/non denatured ethanol. Add **600 µl** of **80 % ethanol** to the Spin Filter and centrifuge for 1 minute at 11,000 x g. Discard the flow through. For removing the ethanol centrifuge for 2 minutes at 11,000 x g. Continue with the elution step as described.
Problems with mineral oil	Increase volume of **Binding Buffer**.

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