Instructions for Use
Life Science Kits & Assays

innuPREP Gel Extraction Lite Kit

Innuscreen GmbH

Order No.:
845-KS-5031100 100 reactions

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Contents

1 Introduction

1.1 Intended use

The innuPREP Gel Extraction Lite Kit has been designed as an extremely fast, simple and highly efficient method for extraction and concentration of DNA fragments from TAE or TBE agarose gels

The kit uses an optimized chemistry resulting in a very fast and reliable purification of DNA fragments and a high rate of recovery.

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	**REF** Catalogue number.
Σ N	**Content** Contains sufficient reagents for <N> tests.
30 °C 15 °C	**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	**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 Gel Extraction 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. Or take care not to carry them over into the sample preparation procedure. 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 Gel Extraction Lite 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 (→ “Intended use” p. 2) (→ “Product specifications” p. 8). Since the performance characteristics of our kits have not been validated for any specific application. 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 equivalents 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 Included kit components

	Σ 100
REF	845-KS-5031100
Gel Solubilizer	75 ml
Binding Optimizer	10 ml
Washing Solution LS (conc.)	36 ml
Spin Filter	10
Receiver Tubes	10
Manual	1

Components not included in the kit

- 1.5 ml and 2.0 ml reaction tubes
- Ethanol (96–99.8 %); non denatured or methylated
- Ultrapure Water/ddH_2O or Elution Buffer (10 mM Tris, pH 8.0 – 8.5)

7 Product specifications

1. Starting material:
   - TAE agarose gels (up to 300 mg)
   - TBE agarose gels (up to 300 mg)

2. Time for isolation:
   - Approximately 20 minutes

3. Binding capacity and fragment length:
   - Binding capacity: > 20 µg DNA
   - Fragment length: 100 bp–30 kbp

4. Rate of recovery:
   - 60–90 % (depending on the length of DNA amplicons)

8 Initial steps before starting

- Add 144 ml of absolute ethanol to **Washing Solution LS (conc.)**, mix thoroughly and store as described above. Keep the bottle firmly closed!
- Heat thermal mixer or water bath to 50 °C.
- Centrifugation steps should be carried out at room temperature.
- Centrifugation steps should be carried out at room temperature.
- Optionally, heat the needed amount of **Elution Buffer (10 mM Tris, pH 8.0 – 8.5)** to 50 °C. The final elution step with heated **Elution Buffer** will increase the DNA yield!

9 Standard protocol: DNA extraction from agarose gel slices (TAE or TBE agarose gels)

NOTE

The standard protocol allows the elution of the bound DNA fragment with standard volumes of **Elution Buffer (10 mM Tris, pH 8.0 – 8.5)** between 30–50 µl!

IMPORTANT NOTE!

Optionally, heat the needed amount of **Elution Buffer (10 mM Tris, pH 8.0 – 8.5)** to 50 °C.
The final elution step with heated **Elution Buffer** will increase the DNA yield!

1. Excise the DNA fragment from the agarose gel with a sharp scalpel.

NOTE

Minimize the agarose gel slice. Check the weight. Do not use more than 300 mg gel slice for one Spin Filter!

2. Transfer the gel slice into a 1.5 ml or 2.0 ml reaction tube and add **650 µl Gel Solubilizer**.
3. Incubate for 10 minutes at 50 °C until the agarose gel slice is completely dissolved.

NOTE

We recommend using a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Alternatively, vortex the sample 3–4 times during incubation.

4. Add **50 µl Binding Optimizer** and mix the suspension by vortexing or pipetting sometimes up and down.
5. Apply the sample onto the Spin Filter located in a Receiver Tube. Close the cap and centrifuge at 10,000 x g (~12,000 rpm) for 1 minute.

NOTE

If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

Discard the filtrate and re-use the Receiver Tube. Place the Spin Filter back into the Receiver Tube.

6. Open the Spin Filter and add **700 µl Washing Solution LS**, close the cap and centrifuge at 10,000 x g (~12,000 rpm) for 1 minute. Discard the filtrate and re-use the Receiver Tube. Place the Spin Filter back into the Receiver Tube.
7. Repeat step 6 completely.
8. Centrifuge at max. speed for 2 minutes to remove all traces of ethanol. Discard the Receiver Tube.
9. Place the Spin Filter into an 1.5 ml Elution Tube. Carefully open the cap of the Spin Filter and add **30–50 µl Elution Buffer (10 mM Tris, pH 8.0 – 8.5)** (optionally pre-warmed to 50 °C). Incubate at room temperature for 1 minute. Centrifuge at 6,000 x g (~8,000 rpm) for 1 minute. A second elution step will increase the yield of extracted DNA.

NOTE

The DNA can be eluted with a lower or a higher volume of **Elution Buffer (10 mM Tris, pH 8.0 – 8.5)** (depends on the expected yield of DNA). Elution with lower volumes of **Elution Buffer** increases the final concentration of DNA. Store the extracted DNA at 4–8 °C. For long time storage placing at -22 °C to -18 °C is recommended.

10 "Mini Elute" protocol: DNA extraction from agarose gel slices (TAE or TBE agarose gels)

NOTE

The "Mini Elute" protocol allows the elution of the bound DNA fragment with low volumes of **Elution Buffer (10 mM Tris, pH 8.0 – 8.5)** between 10–20 µl!

IMPORTANT NOTE!

Optionally, heat the needed amount of **Elution Buffer (10 mM Tris, pH 8.0 – 8.5)** to 50 °C.
The final elution step with heated **Elution Buffer** will increase the DNA yield!

1. Excise the DNA fragment from the agarose gel with a sharp scalpel.

NOTE

Minimize the agarose gel slice. Check the weight. Do not use more than 300 mg gel slice for one Spin Filter!

2. Transfer the gel slice into a 1.5 ml or 2.0 ml reaction tube and add **650 µl Gel Solubilizer**.
3. Incubate for 10 minutes at 50 °C until the agarose gel slice is completely dissolved.

NOTE

We recommend using a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Alternatively, vortex the sample 3–4 times during incubation.

4. Add **50 µl Binding Optimizer** and mix the suspension by vortexing or pipetting sometimes up and down.

5. Apply the sample onto the Spin Filter located in a Receiver Tube. Close the cap and centrifuge at 10,000 x g (~12,000 rpm) for 1 minute.

NOTE

If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

Discard the filtrate and re-use the Receiver Tube. Place the Spin Filter back into the Receiver Tube.

6. Open the Spin Filter and add **700 µl Washing Solution LS**, close the cap and centrifuge at 10,000 x g (~12,000 rpm) for 1 minute. Discard the filtrate and re-use the Receiver Tube. Place the Spin Filter back into the Receiver Tube.
7. Repeat step 6 completely.
8. Centrifuge at max. speed for 2 minutes to remove all traces of ethanol. Discard the Receiver Tube.
9. Place the Spin Filter into an Elution Tube. Carefully open the cap of the Spin Filter and add **10–20 µl Elution Buffer (10 mM Tris, pH 8.0 – 8.5)** (optionally pre-warmed to 50 °C). Incubate at room temperature for 2 minutes. Centrifuge at 8,000 x g (~10,000 rpm) for 1 minutes. A second elution step will increase the yield of extracted DNA.

   The recovery rate of the elution volume is approx. 9 µl in case of 10 µl of **Elution Buffer**.

NOTE

The DNA can be eluted with a lower or a higher volume of **Elution Buffer (10 mM Tris, pH 8.0 – 8.5)** (depends on the expected yield of DNA). Elution with lower volumes of **Elution Buffer** increases the final concentration of DNA. Store the extracted DNA at 4–8 °C. For long time storage placing at -22 °C to -18 °C is recommended.

11 Troubleshooting

Problem / probable cause	Comments and suggestions
Low recovery / poor quality	
Incorrect **Washing Solution LS** or no ethanol added	Prepare the Washing Solution LS exactly as described in the manual. Store the Washing Solution with firmly fixed cap
Poor elution of DNA	Add the Elution Buffer (10 mM Tris, pH 8.0 – 8.5) directly onto the center of the Spin Filter (even if a small elution volume is used).
Ineffective solubilization of the agarose gel slice	The gel slice must be completely dissolved.
No **Binding Optimizer** added	Add the amount of Binding Optimizer needed to the solubilized suspension.
Problems with downstream application, e.g. ligation	
Contamination with salt components	Wash the Spin Filter as described in the manual.
Contamination with agarose traces	Wash the Spin Filter once with Gel Solubilizer.
Contamination of the final DNA with ethanol	Keep the given centrifugation time, extend it if necessary (test the smell).

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