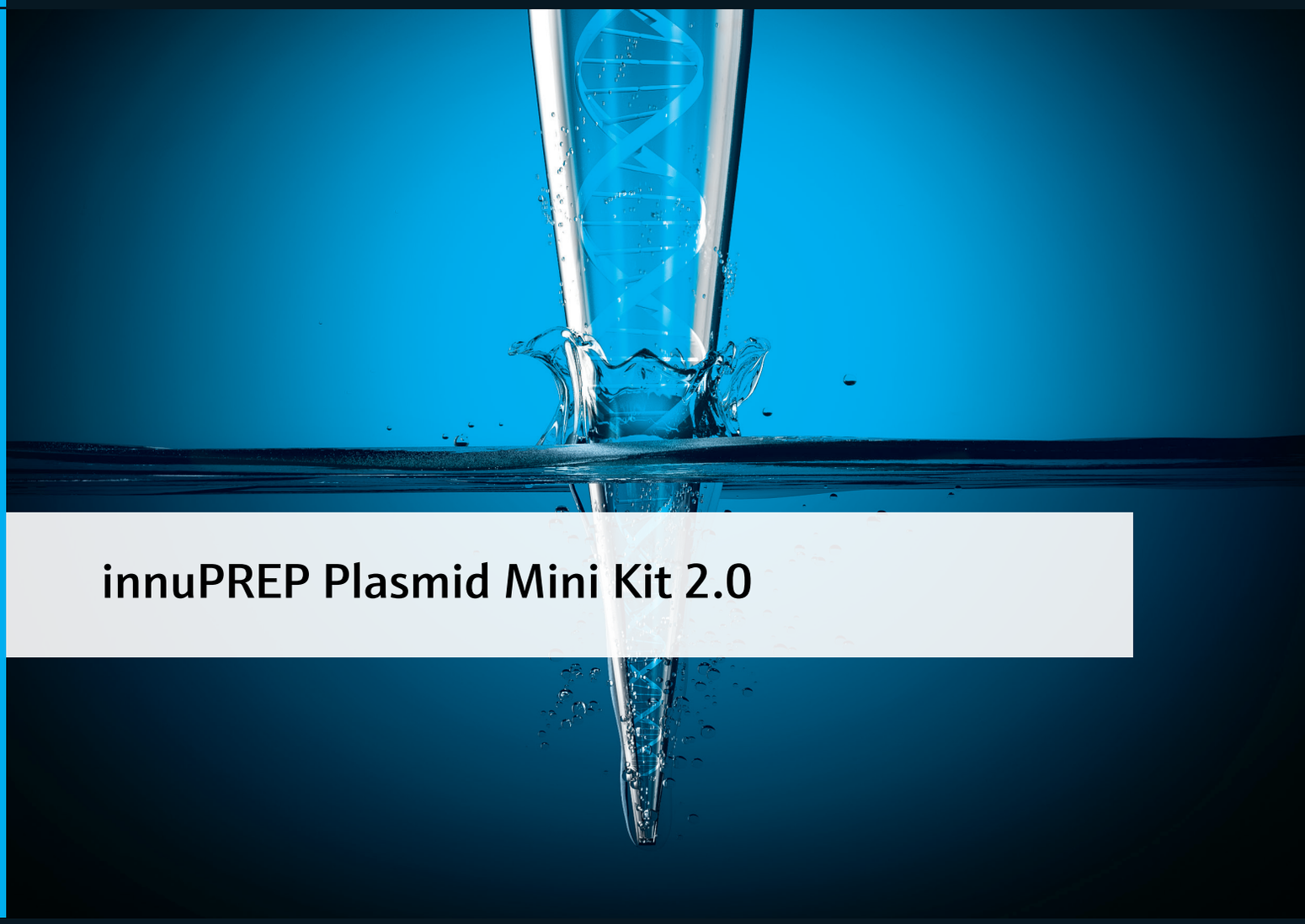
Instructions for Use
Life Science Kits & Assays



innuPREP Plasmid Mini Kit 2.0

Innuscreen GmbH

Order No.:

845-KS-5041010 10 reactions
845-KS-5041050 50 reactions
845-KS-5041250 250 reactions
845-KS-5041500 500 reactions

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This documentation describes the state at the time of publishing.
It needs not necessarily agree with future versions. Subject to change!

Manufacturer and Distributor:
IST Innuscreen GmbH
Robert-Rössle-Straße 10
13125 Berlin · Germany
Made in Germany!

Phone +49 30 9489 3380
Fax +49 30 9489 3381
info.innu@ist-ag.com

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1 Introduction

1.1 Intended use

The innuPREP Plasmid Mini Kit 2.0 has been designed for the extraction of plasmid DNA from up to 15 ml of cultured bacterial cells. The kit uses an optimized chemistry in combination with a new designed spin filter material for fast and reliable purification of plasmid DNA and for high yield of plasmid DNA.

The innuPREP Plasmid Mini Kit 2.0 protocol is based on an alkaline lysis procedure combined with binding of plasmid DNA (pDNA) on the surface of a spin filter column. After binding the pDNA is washed to remove RNA, proteins or other impurities. The eluted plasmid DNA is from excellent quality and therefore highly suited for a lot of downstream applications like transfection, cloning, sequencing, PCR or in vitro transcription.

CONSULT INSTRUCTION FOR USE

This package insert must be read carefully prior to 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
15 °C 30 °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 prior 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 is to 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 Plasmid Mini Kit 2.0 should be stored dry at room temperature (15 °C–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 Plasmid Mini Kit 2.0 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

6.1 Included kit components

	Σ 10	Σ 50
REF	845-KS-5041010	845-KS-5041050
Resuspension Buffer	6 ml	30 ml
Lysis Buffer	6 ml	30 ml
Neutralization Buffer	8 ml	40 ml
Washing Solution A	8 ml	40 ml
Washing Solution B (conc.)	4 ml	20 ml
Elution Buffer P	2 ml	3 x 2 ml
Spin Filter	10	50
Receiver Tubes	10	50
Manual	1	1

	Σ 250	Σ 500
REF	845-KS-5041250	845-KS-5041500
Resuspension Buffer	150 ml	2 x 150 ml
Lysis Buffer	150 ml	2 x 150 ml
Neutralization Buffer	200 ml	2 x 200 ml
Washing Solution A	180 ml	2 x 180 ml
Washing Solution B (conc.)	80 ml	2 x 80 ml
Elution Buffer P	30 ml	2 x 30 ml
Spin Filter	5 x 50	10 x 50
Receiver Tubes	5 x 50	10 x 50
Manual	1	1

6.2 Components not included in the kit

- 1.5 ml or 2.0 ml reaction tubes or 15 ml reaction tubes (optional)
- 96–99.8 % ethanol ; non denatured or methylated

7 Product specifications

1. Starting material:
   - Bacterial culture (0.5–15.0 ml) for isolation of high copy plasmid DNA
   - Bacterial culture (0.5–15.0 ml) for isolation of low copy plasmid DNA
   - Bacterial culture (0.5–15.0 ml) for isolation of cosmid DNA

2. Time for isolation:
   - Approximately 15 minutes

3. Binding capacity and typical yield:
   - Typical yield from 2.0 ml starting material: 6–20 µg (high copy plasmid)
   - Typical yield from 15.0 ml starting material: 45–60 µg (high copy plasmid)

8 Initial steps before starting

- Add the indicated amount of absolute ethanol to **Washing Solution B (conc.)**, mix thoroughly and store as described above.

845-KS-5041010	Add 6 ml ethanol to 4 ml Washing Solution B (conc.).
845-KS-5041050	Add 30 ml ethanol to 20 ml Washing Solution B (conc.).
845-KS-5041250	Add 120 ml ethanol to 80 ml Washing Solution B (conc.).
845-KS-5041500	Add 120 ml ethanol to 80 ml Washing Solution B (conc.).

- Centrifugation steps should be carried out at room temperature.

9 Protocol 1: Isolation of plasmid DNA from 0.5–5 ml bacterial culture

1. Transfer **0.5 ml up to 5 ml** of the overnight *E. coli* culture into a 1.5 ml, 2.0 ml or 15 ml reaction tube. Centrifuge for 1 minute at maximum speed to pellet the bacteria; remove the supernatant as completely as possible.
2. Resuspend the bacterial cell pellet in **250 µl Resuspension Buffer** completely by vortexing or by pipetting up and down.

NOTE

No bacterial cell pellet or clumps should be visible.

3. Add **250 µl Lysis Buffer**, close the tube and mix carefully by inverting the tube 6–8 times. Do not perform the lysis step longer than 5 minutes.

ATTENTION

Please check **Lysis Buffer** for precipitates. If white precipitates are visible, warm the **Lysis Buffer** several minutes at 30 °C–40 °C until the precipitates are dissolved. Cool **Lysis Buffer** down to room temperature.

IMPORTANT

Don't vortex the tube to mix the suspension! This step is critical for the separation of bacterial chromosomal DNA from plasmid DNA. Mechanical stress by vortexing or extensive mixing leads to shearing of high-molecular weight chromosomal DNA. This sheared chromosomal DNA is not precipitated by NaOH/SDS and contaminates the plasmid DNA.

4. Add **350 µl Neutralization Buffer** and mix gently, but thoroughly by inverting the tube 6–8 times. Centrifuge for 8 minutes at full speed (12,000–14,000 rpm). During centrifugation place the needed amounts of Spin Filters into 2.0 ml Receiver Tubes.

NOTE

Lysate and Neutralization Buffer have to be completely homogenous. An inhomogeneous solution leads to dramatically loss of final yield of pDNA.

5. Apply the **clarified supernatant** onto the Spin Filter located in a 2.0 ml Receiver Tube. Centrifuge at 11,000 x g (~11,000 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 2.0 ml Receiver Tube. Place the Spin Filter back into the 2.0 ml Receiver Tube.

6. Add **500 µl Washing Solution A** to the Spin Filter and centrifuge at 11,000 x g (~11,000 rpm) for 1 minute. Discard the filtrate and re-use the 2.0 ml Receiver Tube. Place the Spin Filter back into the 2.0 ml Receiver Tube.
7. Add **700 µl Washing Solution B** to the Spin Filter and centrifuge at 11,000 x g (~11,000 rpm) for 1 minute.
8. Discard the filtrate after the washing step and re-use the 2.0 ml Receiver Tube. Place the Spin Filter back into the 2.0 ml Receiver Tube. Centrifuge at full speed (12,000–14,000 rpm) for 2 minutes to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.
9. Place the Spin Filter into a 1.5 ml reaction tube (not provided) and add **50–100 µl Elution Buffer P** onto the center of the Spin Filter. Incubate at room temperature for 1 minute. Centrifuge at 11,000 x g (~11,000 rpm) for 1 minute. Dividing the final elution volume in two equal volumes of Elution Buffer P increases the final concentration of pDNA in the first elution step, but not the yield of eluted pDNA.

NOTE

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

10 Protocol 2: Isolation of plasmid DNA from 5–15 ml bacterial culture

1. Transfer **5 ml up to 15 ml** of the overnight *E. coli* culture into a 15 ml reaction tube. Centrifuge for 8 minutes at maximum speed to pellet the bacteria; remove supernatant as completely as possible.
2. Resuspend the bacterial pellet in **550 µl Resuspension Buffer** completely by vortexing or by pipetting up and down. Transfer the resulting solution into a 2.0 ml reaction tube.

NOTE

No bacterial cell pellet or clumps should be visible.

3. Add **550 µl Lysis Buffer**, close the tube and mix carefully by inverting the tube 6–8 times and incubate the sample at room temperature for 5 minutes. Do not perform the lysis step longer than 5 minutes.

ATTENTION

Please check **Lysis Buffer** for precipitates. If white precipitates are visible, warm the **Lysis Buffer** several minutes at 30–40 °C until the precipitates are dissolved. Cool **Lysis Buffer** down to room temperature.

IMPORTANT

Don't vortex the tube to mix the suspension! This step is critical for the separation of bacterial chromosomal DNA from plasmid DNA. Mechanical stress by vortexing or extensive mixing leads to shearing of high-molecular weight chromosomal DNA. This sheared chromosomal DNA is not precipitated by NaOH/SDS and contaminates the plasmid DNA.

4. Add **750 µl Neutralization Buffer** and mix gently, but thoroughly by inverting the tube 6–8 times. Centrifuge for 8 minutes at full speed (12,000–14,000 rpm). During centrifugation place the needed amounts of Spin Filters into 2.0 ml Receiver Tubes.

NOTE

Lysate and Neutralization Buffer have to be completely homogenous. An inhomogeneous solution leads to dramatically loss of final yield of pDNA.

5. Apply **850 µl** of the **clarified supernatant** onto the Spin Filter located in a 2.0 ml Receiver Tube. Centrifuge at 11,000 x g (~11,000 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.

6. Discard the filtrate and re-use the 2.0 ml Receiver Tube. Apply the **residual sample** onto the Spin Filter located in the 2.0 ml Receiver Tube. Centrifuge at 11,000 x g (~11,000 rpm) for 1 minute. Discard the filtrate and re-use the 2.0 ml Receiver Tube (place the Spin Filter back into the 2.0 ml Receiver Tube).
7. Add **650 µl Washing Solution A** to the Spin Filter and centrifuge at 11,000 x g (~11,000 rpm) for 1 minute. Discard the filtrate and re-use the 2.0 ml Receiver Tube. Place the Spin Filter back into the 2.0 ml Receiver Tube.
8. Add **750 µl Washing Solution B** to the Spin Filter and centrifuge at 11,000 x g (~11,000 rpm) for 1 minute.
9. Discard the filtrate after the washing step and re-use the 2.0 ml Receiver Tube. Place the Spin Filter back into the 2.0 ml Receiver Tube. Centrifuge at full speed (12,000–14,000 rpm) for 3 minutes to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.
10. Place the Spin Filter into a 1.5 ml reaction tube (not provided) and add **100 µl Elution Buffer P** onto the center of the Spin Filter. Incubate at room temperature for 3 minutes. Centrifuge at 11,000 x g (~11,000 rpm) for 1 minute. Two elution steps with an equal volume of **Elution Buffer P** will increase the yield of extracted plasmid DNA.

NOTE

The DNA can be eluted with a lower or a higher volume of **Elution Buffer P** (depends on the expected yield of plasmid DNA). Elution with lower volumes of **Elution Buffer P** increases the final concentration of pDNA. 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	
Incorrect **Washing Solution B** or no ethanol added	Prepare the **Washing Solution B** exactly as described in the manual. Store the **Washing Solution B** with firmly fixed cap.
Poor elution of pDNA	Add the **Elution Buffer P** directly onto the center of the Spin Filter (even if a small elution volume is used).
Ineffective resuspension or lysis of bacteria cells	The cell pellet must be completely resuspended. After addition of **Lysis Buffer**, the solution should become clear. Increase time for lysis up to 5 minutes.
Incorrect neutralization	Do not shake or vortex the sample after adding **Neutralization Buffer**. Mix by inverting the tube minimal 6–8 times.
Problems with down-stream application, e.g. ligation	
Contamination with salt components	Wash the Spin Filter as described in the manual.
Contamination of the final DNA with ethanol	Keep the given centrifugation time, extend it if necessary (test the smell).

IST Innuscreen GmbH
Robert-Rössle-Str.10
13125 Berlin · Germany

Phone +49 30 9489 3380
Fax +49 30 9489 3381

info.innu@ist-ag.com

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