Instructions for Use
Life Science Kits & Assays

innuPREP Plasmid Mini Kit 2.0

analytikjena
An Endress+Hauser Company

Order No.:
845-KS-5041010 10 reactions
845-KS-5041050 50 reactions
845-KS-5041250 250 reactions
845-KS-5041500 500 reactions

Publication No.: HB_KS-5041_e_210401

Manufacturer:
AJ Innuscreen GmbH
Robert-Rössle-Straße 10
13125 Berlin
Made in Germany!

Distribution/Publisher:
Analytik Jena GmbH
Konrad-Zuse-Straße 1
07745 Jena · Germany

Phone +49 3641 77 9400
Fax +49 3641 77 767776
www.analytik-jena.com
info@analytik-jena.com

Contents

1 Introduction

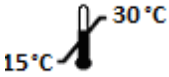
1.1 Intended use

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.

1.2 Notes on the use of this manual

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

Symbol	Information
	**REF** Catalogue number
 N	**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 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.

Clinical sample must always be considered as potentially infectious. Samples from risk patients must always be labeled and handled under consequent safety conditions. Please observe the federal, state and local safety and environmental regulations. Observe 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, please ask for the material safety data sheet (MSDS).

3 Storage conditions

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 Analytik Jena 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 Analytik Jena GmbH products, please do not hesitate to contact us. For technical support or further information in Germany please dial +49 36 41 / 77 94 00. For other countries please contact your local distributor.

5 Product use and warranty

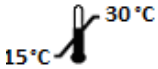
The kit is not designed for the usage of other starting materials or other amounts of starting materials than those, referred to in the manual (→ “Intended use” p.2) (→ “Product specifications” p.9). Since the performance characteristics of Analytik Jena GmbH kits have just been validated for the application described above, the user is responsible for the validation of the performance of Analytik Jena GmbH kits using other protocols than those described below. Analytik Jena 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 Analytik Jena 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

For research use only!

6 Kit components



STORAGE CONDITIONS

All components are stored at room temperature.

	Σ 10	Σ 50
Spin Filter with cap	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
Initial steps	**Washing Solution B** Add 6 ml of 96-99.8 % ethanol to the bottle and mix thoroughly. Keep the bottle always firmly closed!	**Washing Solution B** Add 30 ml of 96-99.8 % ethanol to the bottle and mix thoroughly. Keep the bottle always firmly closed!!

	Σ 250	Σ 500
Spin Filter with cap	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
Initial steps	**Washing Solution B** Add 120 ml of 96-99.8 % ethanol to the bottle and mix thoroughly. Keep the bottle always firmly closed!	**Washing Solution B** Add 120 ml of 96-99.8 % ethanol to the bottle and mix thoroughly. Keep the bottle always firmly closed!!

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

NOTE

Use only absolute/pure ethanol, NO methylated or denatured alcohol!

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 Recommended steps before starting

- Ensure that the **Washing Solution B** has been prepared according to the instruction (→ "Kit components" p.7).
- Centrifugation steps should be carried out at room temperature.

9 General procedure for DNA extraction

Alkaline lysis of the starting material

Clearing supernatant by centrifugation

Binding of pDNA onto Spin Filter

Washing of the bound pDNA

Elution of pDNA

10 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.

11 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.

12 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).

Headquarters

Analytik Jena GmbH
Konrad-Zuse-Str. 1
07745 Jena · Germany

Phone +49 3641 77 70
Fax +49 3641 77 9279
info@analytik-jena.com
www.analytik-jena.com

Pictures: Analytik Jena GmbH
Subject to changes in design and scope of delivery as well as further technical development!

analytikjena
An Endress+Hauser Company