Instructions for Use
Life Science Kits & Assays

innuPREP Plasmid Midi Kit

iST Innuscreen GmbH

Order No.:
845-KS-5043010 10 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!

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1 Introduction

1.1 Intended use

The innuPREP Plasmid Midi Kit has been designed for the extraction of plasmid DNA from up to 100 ml of cultured bacterial cells. The kit uses an optimized chemistry with an increased efficiency for low-and high-copy plasmid DNA purification combined with an efficient endotoxin removal and a DNA precipitation procedure.

The precipitated 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 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
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 and the kit" 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 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.

Upon arrival, store **Spin Filter Midi** and **Resuspension Solution Midi** at 4 °C to 8 °C.

All other components of the innuPREP Plasmid Midi Kit 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.

4 Functional testing and technical assistance

The IST Innuscreen GmbH guarantees the correct function of the kit for applications as described in the manual.

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 Midi 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

6.1 Included kit components

	Σ 10
REF	845-KS-5043010
Removal Solution Midi	15 ml
Resuspension Solution Midi	55 ml
Lysis Solution Midi	55 ml
Neutralization Solution Midi	55 ml
Washing Solution Midi	220 ml
Elution Buffer Midi	90 ml
TE Buffer Midi	16 ml
Precipitation Buffer Midi	350 µl
Isopropanol Midi	60 ml
Prefilter Midi	10
Spin Filter Midi	10
50 ml Tubes	10
Counterweight	1
Manual	1

6.2 Components not included in the kit

- 15 ml and 50 ml sterile falcon tubes
- 70 % ethanol (non-denatured or methylated)
- Sterile water (nuclease-free); optional

7 Product specifications

1. Starting material:
   - Bacterial culture (up to 100 ml) for isolation of high copy plasmid DNA
   - Bacterial culture (up to 100 ml) for isolation of low copy plasmid DNA

2. Time for isolation:
   - Approximately 90 minutes

3. Binding capacity:
   - 200 µg DNA

8 Protocol 1: Isolation of plasmid DNA from 100 ml bacterial culture

1. Centrifuge up to 100 ml of overnight *E. coli* culture. Remove the supernatant
2. Resuspend the bacterial pellet in 5 ml of **Resuspension Solution Midi**.

NOTE
During the pellet resuspension, the solution will change color from transparent deep pink to opaque light pink. The resuspension complete when the pellet at the bottom of the tube has disappeared completely.

3. Add **5 ml Lysis Solution Midi**, close the tube, and mix carefully by inverting the tube. Incubate for 5 minutes at 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.

NOTE
The mixture should change appearance and color. After 3 minutes of incubation, the lysate must be completely clear and uniformly raspberry color. If not, mix the lysate a few times and incubate again for 3 minutes at room temperature

4. Add **5 ml Neutralization Solution Midi** and mix gentlyuntil the raspberry color of the lysate disappears.

NOTE
The addition of Neutralization Solution Midi results in the rapid precipitation of the potassium salts (SDS), chromosomal DNA and certain proteins.

After mixing, the tube content should turn yellowish. When no traces of raspberry color are visible, neutralization is complete and alkaline lysis has been successfully completed.

5. Transfer the lysate onto the **Prefilter Midi**. Close the tube and centrifuge for 5 minutes at 1500 x g
6. Add **1,2 ml Removal Solution Midi** to the filtered lysate. Mix and leave on ice for 30 minutes.
7. Insert **Spin Filter Midi** to a **50 ml Tube.**
8. **Transfer the lysate from Point 6 to the Spin Filter Midi. Wait for the lysate to flow through the filter.**
9. Add **20 ml Washing Solution Midi** to the **Spin Filter Midi** and wait for the solution to flow through the filter.
10. Transfer the Spin Filter Midi to the new 50 ml Falcon Tube (not included).
11. Add **6 ml Elution Buffer Midi**. Wait for the eluate to flow through the filter.
12. Transfer the eluate to a new 15 ml Falcon Tube (not included).
13. Add **25 µl Precipitation Buffer Midi** and **5 ml Isopropanol Midi** to the eluate.

NOTE

In cases where the addition of a Precipitation Buffer Midi is not necessary or not desired (e.g., very sensitive transfection), 5 ml of isopropanol should be added only. This will not affect the isolation efficiency.

14. Mix the sample by inverting the tube a few times and centrifuge for **10 minutes at 11 000 x g.**
15. Pour out the supernatant from the tube. Be careful not to remove the DNA pellet at the bottom of the tube.

ATTENTION

When pouring out the supernatant, it is very easy to lose the DNA pellet. For safety, it is recommended to pour out the supernatant into the prepared tube so the pellet can be recovered.

16. Add **2 ml 70% ethanol** (not included). Mix the sample and centrifuge for **3 minutes at 11 000 x g**.
17. Pour out supernatant of the tube. Be careful not to remove the DNA pellet at the bottom of the tube.

NOTE

The light-blue colored DNA pellet should be visible at the bottom of the precipitation tube.

18. Invert the tube with the plasmid DNA pellet and air-dry for **10 minutes at room temperature.**

NOTE

If there are any leftovers (small droplets) of alcohol on the tube walls they should be removed with sterile cotton buds.

19. Dried DNA pellets can be dissolved in **0,2 – 1 ml TE Buffer Midi** or sterile water (not included).

NOTE

The blue color of the DNA precipitate enables visual confirmation of the DNA dissolution process.

20. Store the plasmid DNA at 4°C to 8°C.

9 LySee color system

LySee color system enables an easy and convenient visual control of alkaline lysis. The visual control system prevents common handling errors of incomplete cell resuspension, inefficient cell lysis and incomplete precipitation of unwanted cell components.

Resuspension and lysis

The addition of the transparent purple colored Resuspension Solution Midi to the bacterial cell pellet makes the bacterial cell pellet easy to localize (fig 1). During the suspension of the bacterial cell pellet, the solution turns opaque light pink (fig 2). The suspension is completed with the complete disappearance of the pellet at the bottom of the tube. After the addition of Lysis Solution Midi and incubation, the lysate turns transparent raspberry. Cell lysis is completed when the solution will turn homogeneously transparent raspberry (fig 3).

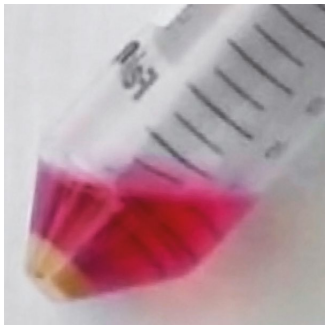

fig 1

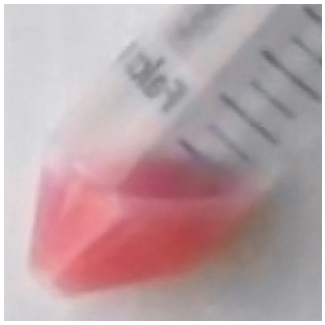

fig 2

fig 3

Neutralization and precipitation

The addition of the Neutralization Solution Midi causes rapid precipitation of potassium salts (SDS), chromosomal DNA and some proteins (fig 4). After mixing, the solution turns yellowish (fig 5). No traces of raspberry color indicate complete neutralization and successful ending of alkaline lysis (fig 6)

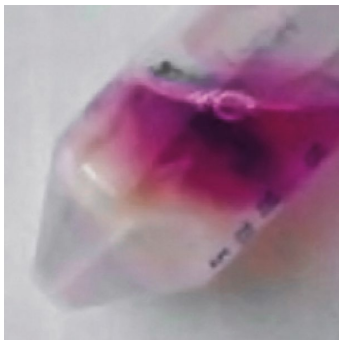

fig 4

fig 5

fig 6

10 Troubleshooting

Problem / probable cause	Comments and suggestions
Low recovery	
Ineffective resuspension or lysis of bacteria cells	The cell pellet must be completely resuspended. After addition of **Lysis Buffer Midi**, the solution should become clear and uniformly raspberry colored. Increase time for lysis up to 3 minutes.
Incorrect neutralization	Do not shake or vortex the sample after adding **Neutralization Buffer Midi**. Mix by inverting the tube several times until the color changed to yellowish.
Problems with down-stream application, e.g. ligation	
Problems with down-stream application, e.g. ligation	Wash the Spin Filter as described in the manual.
Contamination of the final DNA with ethanol	Extend the drying time after the 70% Ethanol wash step.

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