

Life science unlimited

Manual



innuPREP Plasmid Mini Kit

Publication No.: HB_KS-5040_e_080605

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1 Safety precautions

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.

2 Storage conditions

The innuPREP Plasmid Mini Kit should be stored dry, at room temperature (14-25 °C) and is stable for at least 12 months under these conditions. Before every use make sure that all components have room temperature. If there are any precipitates within the provided solutions solve these precipitates by careful warming.

3 Function testing and technical assistance

The Analytik Jena AG guarantees the correct function of the kit for applications as described in the manual. The components of each innuPREP Plasmid Mini Kit were tested by isolation of Plasmid DNA and subsequent analysis of extracted Plasmid DNA.

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 or other Analytik Jena AG 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.

4 Product use and warranty

The user is responsible to validate the performance of the Analytik Jena AG kits for any particular use, since the performance characteristics of our kits have not been validated for any specific application. Analytik Jena AG 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 Analytik Jena AG 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!

5 Kit components



Important




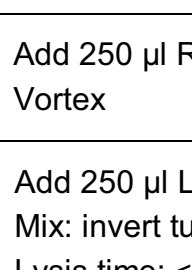
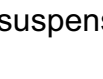

Store all components at room temperature.

	10 extractions	50 extractions	250 extractions
Resuspension Buffer	2 x 2 ml	15 ml	75 ml
Lysis Buffer	2 x 2 ml	15 ml	75 ml
Neutralization Buffer	2 x 2 ml	20 ml	100 ml
Washing Solution A	6 ml	30 ml	140 ml
Washing Solution B	4 ml (final volume 10 ml)	20 ml (final volume 50 ml)	80 ml (final volume 200 ml)
Elution Buffer P	2 ml	2 x 2 ml	15 ml
Spin Filter	10	50	5 x 50
Receiver Tubes (2.0 ml)	10	50	5 x 50
Elution Tubes (1.5 ml)	10	50	5 x 50
Manual	1	1	1
Initial steps	<ul style="list-style-type: none"> • Add 6 ml of 96-99.8 % ethanol to the bottle Washing Solution B, mix thoroughly and keep the bottle always firmly closed! 	<ul style="list-style-type: none"> • Add 30 ml of 96-99.8 % ethanol to the bottle Washing Solution B, mix thoroughly and keep the bottle always firmly closed! 	<ul style="list-style-type: none"> • Add 120 ml of 96-99.8 % ethanol to the bottle Washing Solution B, mix thoroughly and keep the bottle always firmly closed!

innuPREP Plasmid Mini Kit

Protocol: Isolation of plasmid DNA from bacterial lysates

Recommended steps ▪ Prepare Washing Solution B according to the instruction before starting

1. Starting material		▪ 0.5 – 5.0 ml
2. Pellet cells		▪ Centrifuge: max. speed, 1 min ▪ Discard supernatant
3. Resuspend cells		▪ Add 250 µl Re-suspension Buffer ▪ Vortex
4. Lysis		▪ Add 250 µl Lysis Buffer ▪ Mix: invert tube 5 times ▪ Lysis time: < 5 min
5. Neutralization		▪ Add 350 µl Neutralization Buffer ▪ Mix: shaking tube 4 – 6 times ▪ Centrifuge: max speed, 10 min
6. Binding of DNA		▪ Add Spin Filter to Receiver Tube ▪ Add clarified filtrate to Spin Filter ▪ 10.000 x g (12.000 rpm): 1 min
7. Washing	 Re-use Receiver Tube	▪ Add 500 µl Washing Solution A ▪ 10.000 x g (12.000 rpm): 1 min ▪ Add 700 µl Washing Solution B ▪ 10.000 x g (12.000 rpm): 1 min
8. Remove Ethanol	 Re-use Receiver Tube	▪ Discard filtrate ▪ Add Spin Filter to Receiver Tube ▪ Centrifuge: max speed, 2 min

9. Elution



- Add Spin Filter to an Elution Tube
- Add 30 -100 µl Elution Buffer P
- Incubation: 1 min @ RT
- 6.000 x g (8.000 rpm): 1 min

✂ Cut at the scattered line and laminate the card for a more convenient handling on the table top ✂

Order No.:

845-KS-5040010	10 reactions
845-KS-5040050	50 reactions
845-KS-5040250	250 reactions

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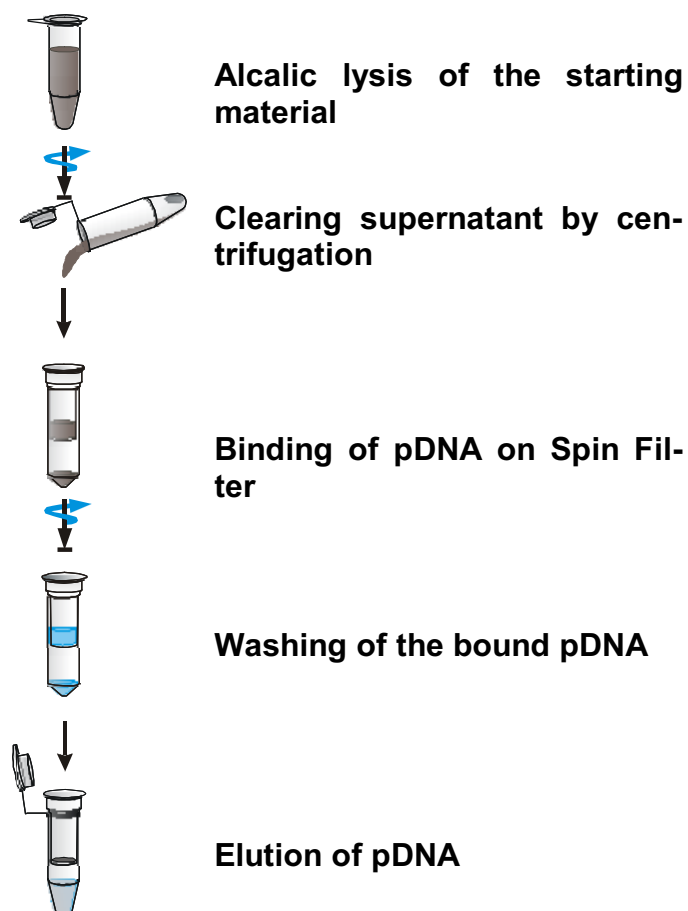
6 Recommended steps before starting

- Ensure that the Washing Solution B has been prepared according to the instruction (→ "Kit components" p. 4)
- Centrifugation steps should be carried out at room temperature

7 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

8 General procedure for DNA extraction



9 Product specifications

1. Starting material:

Bacteria suspension (0.5 – 5.0 ml)

2. Time for isolation:

Approximately 16 minutes

3. Typical yield:

- Approximately 25 µg
- Typical yield from 2.0 ml starting material: 6 – 14 µg

10 Protocol: Isolation of plasmid DNA from 0.5 – 5 ml bacterial lysates

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 cells; remove the supernatant as completely as possible.
2. Re-suspend the bacterial cell pellet in **250 µl Re-suspension Buffer** completely by vortexing or by pipetting up and down.

Note: No cell pellet or clumps should be visible.

3. Add **250 µl Lysis Buffer**, close the tube and mix carefully by inverting the tube 5 times. Do not perform the lysis step more than 5 min.



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 shaking the tube 4-6 times. Centrifuge for 10 minutes at full speed (12.000–14.000 rpm). During centrifugation place the needed amounts of Spin Filters into 2.0 ml Receiver Tubes.
5. Apply the clarified supernatant sample onto the Spin Filter located in a 2.0 ml Receiver Tube. 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 2.0 ml Receiver Tube. Place the Spin Filter into the 2.0 ml Receiver Tube.

6. Add **500 µl Washing Solution A** to the Spin Filter and centrifuge at 10.000 x g (12.000 rpm) for 1 minute. Discard the filtrate and re-use the 2.0 ml Receiver Tube. Place the Spin Filter into the 2.0 ml Receiver Tube.
7. Add **700 µl Washing Solution B** to the Spin Filter and centrifuge at 10.000 x g (12.000 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 into the 2.0 ml Receiver

Tube. Centrifuge at max. speed 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 Elution Tube and add **30-100 μ l Elution Buffer P**. Incubate at room temperature for 1 minute. Centrifuge at 6.000 x g (8.000 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 pDNA at +4 °C. For long time storage placing at -20 °C is recommended.

11 Troubleshooting

Problem / probable cause	Comments and suggestions
<p>Low recovery</p> <ul style="list-style-type: none"> • Incorrect Washing Solution or no ethanol added • Poor elution of pDNA • Ineffective resuspension or lysis of bacteria cells 	<p>Prepare the Washing Solution exactly as described in the manual.</p> <p>Store the Washing Solution with firmly fixed cap.</p> <p>Add the Elution Buffer P directly onto the center of the Spin Filter (even if a small elution volume is used).</p> <p>The cell pellet must be completely re-suspended. After addition of Solution B, the solution should become clear.</p>
<p>Problems with down-stream application, e.g. ligation</p> <ul style="list-style-type: none"> • Contamination with salt components • Contamination of the final DNA with ethanol 	<p>Wash the Spin Filter as described in the manual.</p> <p>Keep the given centrifugation time, extend it if necessary (test the smell).</p>

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