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EliGene® Plasmid DNA MiniPrep
Instructions for Use

Package:

Ref. No.	Quantity
416050	50 Preps

Storage:

All kit reagents and components should be stored at room temperature (15 – 30 °C). When stored under these conditions, the kit will retain full activity until the expiration date indicated on the kit label.

Table of Contents

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Introduction

The EliGene® Plasmid DNA MiniPrep provides a fast, simple and cost-effective plasmid miniprep method for routine molecular biology laboratory applications. EliGene® Plasmid DNA MiniPrep uses alkaline lysis procedure, followed by binding of plasmid DNA silica spin filter to remove proteins and low-molecular–weight impurities. Plasmid DNA isolated with EliGene® Plasmid DNA MiniPrep is immediately ready for use. The entire procedure can be completed in 35 minutes or less, depending on the number of samples processed. All reagents are ready for use and EliGene® Plasmid DNA MiniPrep includes all consumables.

Equipment Required

Microcentrifuge (12,000 x g)
Vortex
Thermostat/Thermoshaker capable of incubation at 60°C
Pipettes: 50 – 750 µl

Kit Contents

Components	Amount (50 isolations)
Resuspension Buffer EP1	12,5 ml
Lysis Buffer EP2	12,5 ml
Neutralization Buffer EP3	17,5 ml
Wash Buffer EP4	25 ml
Wash Buffer EP5	2 x 18 ml
Elution Buffer EP6	4 ml
1.5 ml Tubes	100 pcs
Spin Filters (Units in 2 ml Collection Tubes)	50 pcs

Precautions

Please wear gloves when using this product. Avoid all skin contact with kit reagents. In case of contact, wash thoroughly with water. Do not ingest. See Safety Data Sheets for emergency procedures in case of accidental ingestion or contact.
Reagents labelled flammable should be kept away from open flames and sparks.

WARNING: Wash Buffer EP4 and Wash Buffer EP5 are flammable.

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Detailed Isolation Protocol

It is highly recommended to read these instructions before using EliGene® Plasmid DNA MiniPrep for the first time.

Important Notes Before Using

- **Please always wear gloves.**
- **If there is a precipitate in Resuspension Buffer EP1, Lysis Buffer EP2 or/and in Neutralization Buffer EP3, heat the bottle with buffer to 60 °C to dissolve it.**
- **Removal of residual ethanol from the Spin Filter is critical for efficient elution of DNA by Elution Buffer EP6.**
- **This protocol is intended for the isolation of high-copy plasmid DNA from 1-5 ml overnight cultures.**

1. Centrifuge overnight bacterial cultures 1 minute at 12 000 x g. Remove growth medium as much as possible.

   Note: Residual growth medium could inhibit process of extraction.

2. Completely resuspend bacterial pellet by vortexing in 250 µl of Resuspension Buffer EP1 and transfer it to a 1.5 ml Tube (provided).

   Note: No cell clumps should be visible after resuspension of the pellet.

3. Add 250 µl of Lysis Buffer EP2 and mix by inverting 4-6 times. Incubate at room temperature for 3-4 minutes.

   Note: Do not vortex, because this will result in shearing of genomic DNA. Do not allow the lysis reaction to proceed for more than 5 minutes.

4. Add 350 µl of Neutralization Buffer EP3 and mix by inverting 4-6 times.

   Note: To avoid localized precipitation, mix the solution immediately after addition of Buffer EP3. The solution should become cloudy.

5. Centrifuge for 10 minutes at 12 000 x g.

6. Transfer supernatant to a Spin Filter and centrifuge at 12 000 x g for 1 minute at room temperature.

   Background: Plasmids bind to the silica membrane in the Spin Filter because it is in a chaotropic salt condition. The liquid flow through contains unbound cell material.

7. Remove the Spin Filter and discard the flow through. Place the Spin Filter back into the same 2 ml Collection Tube.
8. Add 500 µl of Wash Buffer EP4 to the Spin Filter. Centrifuge for 1 minute at 12 000 x g.
   Background: Wash Buffer EP4 is a salt based wash solution that cleans the plasmids bound to the Spin Filter from other impurities.
9. Remove the Spin Filter and discard the flow through. Place the Spin Filter back into the same 2 ml Collection Tube.
10. Add 750 µl of Wash Buffer EP5 to the Spin Filter. Centrifuge for 1 minute at 12 000 x g.
    Background: Wash Buffer EP5 is a salt based wash solution that cleans the plasmids bound to the Spin Filter from other impurities.
11. Remove the Spin Filter and discard the flow through. Place the Spin Filter back into the same 2 ml Collection Tube.
12. Centrifuge again for 2 minutes at 12 000 x g to completely dry the Spin Filter membrane.
    Background: The Spin Filter is completely dried of ethanol residues for maximal plasmids release from the Spin Filter membrane in elution step. Residual ethanol from Buffer EP4 and EP5 may inhibit subsequent enzymatic reactions.
13. Carefully transfer the Spin Filter into a new 1.5 ml Tube (provided).
14. Add 50 µl of Elution Buffer EP6 directly to the Spin Filter membrane.
15. Incubate the Spin Filter at room temperature for 1 minute. Centrifuge 1 minute at 10 000 x g.
    Note: To increase yields, incubate for 5 minutes at 60 °C.
16. Remove the Spin Filter unit. Plasmid DNA in tube is now ready to use in any application.

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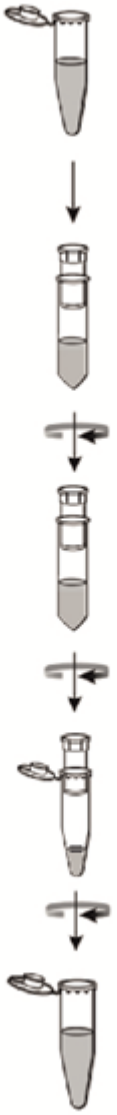
Brief Isolation Protocol



1. Centrifuge overnight bacterial culture 1 minute at 12 000 x g. Remove growth medium as much as possible.
2. Resuspend bacterial pellet in 250 µl Resuspension Buffer EP1 and transfer it to a 1.5 ml Tube (provided).
3. Add 250 µl of Lysis Buffer EP2 and mix by inverting 4-6 times. Incubate at room temperature for 3-4 minutes.
4. Add 350 µl of Neutralization Buffer EP3 and mix by inverting 4-6 times.
5. Centrifuge for 10 minutes at 12 000 x g.
6. Load the supernatant to a Spin Filter and centrifuge at 12 000 x g for 1 minute at room temperature.
7. Remove the Spin Filter and discard the flow through. Place the Spin Filter back into the same 2 ml Collection Tube.
8. Add 500 µl of Wash Buffer EP4 to the Spin Filter. Centrifuge for 1 minute at 12 000 x g.
9. Remove the Spin Filter and discard the flow through. Place the spin filter back into the same 2 ml Collection Tube.
10. Add 750 µl of Wash Buffer EP5 to the Spin Filter. Centrifuge for 1 minute at 12 000 x g.
11. Remove the Spin Filter and discard the flow through. Place the Spin Filter back into the same 2 ml Collection Tube.
12. Centrifuge again for 2 minutes at 12 000 x g to completely dry the Spin Filter membrane.
13. Carefully remove the Spin Filter and transfer it into a new 1.5 ml Tube (provided).
14. Add 50 µl of Elution Buffer EP6 directly to the Spin Filter membrane and incubate at room temperature for 1 minutes.
15. Centrifuge 1 minute at 10 000 x g.
16. Remove the Spin Filter unit. Plasmid DNA in tube is now ready to use in any application.

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Troubleshooting Guide

If DNA Does Not Amplify

- Make sure to check DNA yields and purity by gel electrophoresis, UV spectrophotometer or fluorometer (using PicoGreen®, Molecular Probes, Inc.) reading. An excess amount of DNA would inhibit a PCR reaction.
- Make sure to mix properly Wash Buffers EP4 and EP5 after being unused for a longer time. Components of the buffers may have separated out.
- Dilute the template plasmid.

Eluted DNA Sample Is Colored or Clogging of the Silica Spin Filter

- If you follow recommendations in these instructions, there should not be observed any coloration in isolated DNA.
- Do not use more than 5 ml of overnight bacterial cultures.

Low DNA Yield

- Low yields may be caused by many factors. To find out the the problem, analyze fractions from each step on an agarose gel.
- Take into consideration that different measuring methods result in different concentrations depending whether UV spectrometry of fluorometry is used. Spectrophotometry measures both double-stranded and single-stranded DNA while fluorometry with PicoGreen® (Molecular Probes, Inc.) measures only double-stranded DNA**.**
- Make sure to mix the sample well after adding Lysis Buffer EP2.
- Do not skip the step with removal of residual ethanol from the Spin Filter. It is critical for efficient elution of plasmid from the filter by Elution Buffer EP6.

DNA has Low A260/280 Ratio

The ratio for pure DNA should be 1.7 – 1.9. $A_{260/280}$ reading below 1.6 may signify protein contamination. Low $A_{260/280}$ ratios may be caused by the following:

- If using a Nanodrop, blank the instrument with Elution Buffer EP6.

DNA Floats Out of Well When Loaded on a Gel

- The residues of Wash Buffer EP5 remain in the final sample. Do not skip the step with removal of residual ethanol from the Spin Filter. You may extend the dry spin to 3 minutes or you can use our loading buffer (for more information, please contact our representative).

Concentrating the DNA

The final volume of eluted DNA will be 50 µl. The DNA may be concentrated by adding 10 µl of 3 M sodium acetate (pH = 5.2) and inverting 3 – 5 times to mix. Next, add 200 µl of cold 100% ethanol and invert 3 – 5 times to mix and centrifuge at 12,000 x g for 15 minutes at room temperature. Remove the supernatant and wash the DNA pellet with 70% ethanol. Evaporate residual ethanol in a speed vac, desiccator, or by ambient air and resuspend DNA in desired volume of PCR water or buffer.

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	Catalog number		Upper limit of temperature
	Batch code		Manufacturer
	Use by (last day of month)		Contains sufficient "N" tests