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EliGene® Gel DNA MiniPrep
Instructions for Use

Package:

Ref. No.	Quantity
417050	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® Gel DNA MiniPrep is designed to isolate DNA fragments (80 bp to 10 kbp) from standard or low-melting agarose gel. Depending on DNA fragment size up to 90 % recovery is achieved when DNA is eluted in volume of 30 to 50 µl. EliGene® Gel DNA MiniPrep uses silica spin columns to provide high concentration and quality of DNA isolated from agarose gel slices. DNA isolated with EliGene® Gel DNA MiniPrep is immediately ready for use. The entire procedure can be completed in less than 20 minutes, depending on the number of samples processed. All reagents are ready for use and EliGene® Gel DNA MiniPrep includes all consumables. The buffers provide have been optimized, and do not require monitoring of pH.

Equipment Required

Microcentrifuge (12,000 x g)
Vortex
Thermostat/Thermoshaker
Pipettes: 20 – 750 µl

Kit Contents

Components	Amount (50 isolations)
Gel Solubilization Buffer G1	62,5 ml
Binding Buffer G2	12,5 ml
Wash Buffer G3	37,5 ml
Elution Buffer G4	3 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: Binding Buffer G2 and Wash Buffer G3 are flammable.

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Detailed Isolation Protocol

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

Important Notes Before Using

- **Please always wear gloves.**
- **If there is precipitate in Gel Solubilization Buffer G1, 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 from the filter by Elution Buffer G4.**

1. Excise the DNA fragment from agarose gel with a clean, sharp scalpel.

   Note: Minimize the size of the gel, remove extra agarose from sample.

2. Weigh the gel slice in a 1.5 ml colorless tube (provided) and add 3 volumes of Gel Solubilization Buffer G1 to 1 volume of gel (for example, add 300 µl of Buffer G1 to each 100 mg of gel).

   Note: For ≥ 2 % agarose gels, add 6 volumes of Gel Solubilization Buffer G1. Do not use more than 400 mg gel slice. The total volume of Gel Solubilization Buffer G1 is optimized for 250 mg gel slices, in case of bigger gel slices and/or ≥ 2 % agarose gels, the number of extractions will be reduced.

3. Incubate at 55 °C for 10 minutes or longer until the gel slice has completely dissolved. Mix by vortexing every 3 minutes to help dissolve the gel slice.

4. Add 1 gel volume of Binding Buffer G2 to the sample and mix.

   Note: For example, if the agarose slice is 100 mg, add 100 µl of Binding Buffer G2. Do not centrifuge the sample in this step.

5. Load the sample onto a Spin Filter and centrifuge at 10 000 x g for 1 minute at room temperature.

   Note: The maximum volume for loading is 800 µl. In case of larger volumes, simply load and spin again.

6. Remove the Spin Filter and discard the flow through. Place the Spin Filter back into the same 2 ml Collection Tube.

7. Add 500 µl of Gel Solubilization Buffer G1 to the Spin Filter. Centrifuge for 1 minute at 10 000x g.

   Background: This step will remove all traces of agarose. This step is required if the DNA will be used for direct sequencing, in vitro transcription or microinjection.
8. Remove the Spin Filter and discard the flow through. Place the Spin Filter back into the same 2 ml Collection Tube.
9. Add 750 µl of Wash Buffer G3 to the Spin Filter. Centrifuge for 1 minute at 10 000 x g.

   Background: Wash Buffer G3 is an ethanol wash solution that cleans the DNA bound to the Spin Filter from the other impurities. If the DNA will be used for salt-sensitive applications, let the column stand 3-5 minutes after addition of Wash Buffer G3 before centrifuging.
10. Remove the Spin Filter and discard the flow through. Place the Spin Filter back into the same 2 ml Collection Tubes.
11. Centrifuge again for 2 minutes at 12 000 x g to completely dry the Spin Filter membrane.

    Background: The Spin Filter should be completely dried of ethanol residues for maximal DNA release from the Spin Filter membrane in elution step. Residual ethanol from Wash Buffer G3 may inhibit subsequent enzymatic reactions.
12. Carefully remove the Spin Filter from the 2 ml Collection Tube and transfer it into a new 1.5 ml Tube (provided).
13. Add 50 µl of Elution Buffer G4 directly to membrane of the Spin Filter.

    Note: To increase DNA concentration, add 30 µl of Elution Buffer G4.
14. Incubate at room temperature for 2 minutes. Centrifuge 1 minute at 10 000 x g.

    Note: To increase yields, incubate for 5 minutes at 65 °C.
15. Remove the Spin Filter unit. DNA in the tube is now ready to use in any application.

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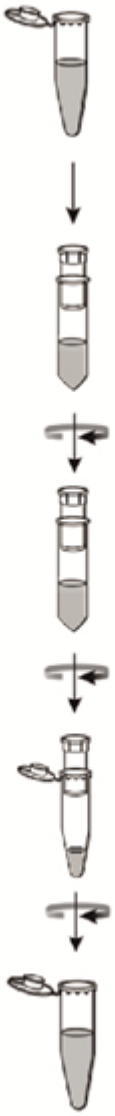
Brief Isolation Protocol



1. Excise the DNA fragment from agarose gel with a clean, sharp scalpel.
2. Weigh the gel slice in a 1.5 ml colorless tube (provided) and add 3 volumes of Gel Solubilization Buffer G1 to 1 volume of gel.
3. Incubate at 55 °C for 10 minutes or longer until the gel slice has completely dissolved, vortex every 3 minutes.
4. Add 1 gel volume of Binding Buffer G2 to the sample and mix.
5. Load the sample to a Spin Filter and centrifuge at 10 000 x g for 1 minute at room temperature.
6. Remove the Spin Filter and discard the flow through. Place the Spin Filter back into the same 2 ml Collection Tubes.
7. Add 500 µl of Gel Solubilization Buffer G1 to the Spin Filter. Centrifuge for 1 minute at 10 000 x g.
8. Remove the Spin Filter and discard the flow through. Place the Spin Filter back into the same 2 ml Collection Tube.
9. Add 750 µl of Wash Buffer G3 to the Spin Filter. Centrifuge for 1 minute at 10 000 x g.
10. Remove the Spin Filter and discard the flow through. Place the Spin Filter back into the same 2 ml Collection Tubes.
11. Centrifuge again for 2 minutes at 12 000 x g to completely dry the Spin Filter membrane.
12. Carefully remove the Spin Filter and transfer it into a new 1.5 ml Tube (provided).
13. Add 50 µl of Elution Buffer G4 and incubate at room temperature for 2 minutes.
14. Centrifuge 1 minute at 10 000 x g.
15. Remove the Spin Filter unit. DNA in tube is now ready to use in any application.

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Troubleshooting Guide

Low or no recovery

- After adding Gel Solubilization Buffer G1 to the gel slice, vortex the tube every 2-3 minutes during the 55°C incubation period. DNA will remain in any undissolved agarose.
- 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 Gel Solubilization Buffer G1 and Binding Buffer G2.
- Do not skip the step with removal of residual ethanol from Spin Filter. It is critical for efficient elution of plasmid from the filter by Elution Buffer G4.
- Approximately 80-90 % recovery can be obtained only from gel slice ≤ 400 mg.

DNA does not perform well in downstream applications

- High salt concentration in eluate. Modify the wash step by incubating the column for 5 min at room temperature after adding 750 µl of Wash Buffer G3.
- Eluate contains residual ethanol. The residues of Wash Buffer G3 remain in the final sample. Do not skip the step with removal of residual ethanol from the Spin Filter.
- Eluate contaminated with agarose. The gel slice is incompletely solubilized or weighs >400 mg, repeat the procedure

DNA Floats Out of Well When Loaded on a Gel

- The residues of Wash Buffer G3 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 business department).

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