metabion international AG
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Version: 01

mi-Gel Extraction Kit

Cat. No. mi-GEL50 & mi-GEL250

Purification of small-scale DNA using phenol/chloroform extraction or ethanol precipitation is laborious and time-consuming. ***metabion***'s mi-Gel Extraction Kit provides a simple and fast method to extract and isolate DNA fragments (ranging from 100bp to 10kb) from agarose gel without using phenol/chloroform. This system is based on binding of up to 20µg DNA to silica-based membranes in chaotropic salts with average recovery rates of 60-90% for 100-bp to 10-kb DNA fragments.

Downstream Applications

- Restriction digestion
- Ligation and cloning
- PCR & Sequencing
- Transformation
- Transfection
- In vitro transcription
- …

Product Contents

Cat. No.	mi-GEL50	mi-GEL250
Preps	**50**	**250**
GEX Buffer	50ml	250ml
WN Buffer	6ml	30ml
WS Buffer	6ml	30ml
Elution Buffer	5ml	25ml
GP Column	50	250
Collection Tube	50	250
Manual	1	1

Storage Conditions

metabion's mi-Gel Extraction Kit can be stored at room temperature up to 12 months. If precipitate forms in any buffer, incubate at 37°C for 30 minutes to resuspend.

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Protocol

Please read the following notes before starting the procedures.

WARNING: Strong acids and oxidants (e.g. bleach) should not be used together with GEX buffer (would produce cyanide)!

Important Notes

- All buffers need to be mixed well before use!
- Buffers provided in this system contain irritants. Appropriate safety apparels such as gloves and lab coat should be worn.
- All procedures should be performed at room temperature (20-25℃).
- All centrifugation steps should be performed at 10,000 x g or 13,000rpm in a microcentrifuge, unless noted otherwise.
- For long-term storage of the eluted DNA, TE buffer should be used for elution. Since EDTA in TE may affect downstream applications, Elution Buffer (provided) or ddH_2O (pH 7.0-8.5) is preferred for elution of DNA immediately used for further enzymatic reactions.

For mi-GEL50

- Add 24ml of 98-100 % ethanol to WN Buffer bottle when first open.
- Add 24ml of 98-100 % ethanol to WS Buffer bottle when first open.

For mi-GEL250

- Add 120ml of 98-100 % ethanol to WN Buffer bottle when first open.
- Add 120ml of 98-100 % ethanol to WS Buffer bottle when first open.

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I. Gel Fragment Purification by Centrifugation

1. **Use a clean, sharp scalpel or razor blade to excise the gel slice containing the DNA fragment of interest.**
   Minimize the size of the gel slice by removing extra agarose. Cutting the gel slice into small pieces can facilitate dissolution.

2. **Measure the weight of the gel slice (about 50-200mg) and place it into a sterile 1.5-ml or 2-ml centrifuge tube. Add 0.5ml GEX Buffer.**
   When agarose percentage of the gel slice is more than 2 %, add GEX Buffer as 5 volumes of the gel slice (100mg = 0.1ml).

3. **Incubate at 60℃ for 5-10 minutes until the gel is completely dissolved. Invert the tube every 1-2 minutes during incubation.**
   Ensure that the gel has been completely dissolved before proceeding to step 4. If the gel slice has not been completely dissolved, more GEX Buffer should be added.
   To increase DNA recovery rate, especially for fragment sizes <500bp and >4kb, add 0.25 volume of isopropanol to the mixture and mix well.

4. **Place a GP™ Column onto a Collection Tube. Load up to 0.7ml dissolved gel mixture onto the column.**

5. **Centrifuge for 30-60 seconds. Discard the flow-through. Repeat step 4 for the rest of the mixture.**

6. **Wash the column once with 0.5ml of WN Buffer by centrifuging for 30-60 seconds. Discard the flow-through.**

7. **Wash the column once with 0.5ml of WS Buffer by centrifuging for 30-60 seconds. Discard the flow-through.**

8. **Centrifuge the column at full speed for 3-5 minutes to remove residual ethanol.**
   It is important to remove residual ethanol, since it may inhibit subsequent enzymatic reactions.

9. **Place the column onto a new 1.5-ml centrifuge tube. Add 15-30µl of Elution Buffer onto the center of the membrane.**
   For effective elution, make sure that the elution solution is dispensed onto the center of the membrane and is completely absorbed.

10. **Stand the column for 2-3 minutes and centrifuge at full speed for 1-2 minutes to elute DNA.**

11. **Short-term (up to a few weeks), store at 4°C; long-term, store at -20°C. To avoid multiple freeze-thaw cycles, preparation of aliquots is recommended.**

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II. Gel Fragment Purification by Vacuum

1. **Use a clean, sharp scalpel or razor blade to excise the gel slice containing the DNA fragment of interest.**
   Minimize the size of the gel slice by removing extra agarose. Cutting the gel slice into small pieces can facilitate dissolution.

2. **Measure the weight of the gel slice (about 50-200mg) and place it into a sterile 1.5-ml or 2-ml centrifuge tube. Add 0.5ml GEX Buffer.**
   When agarose percentage of the gel slice is more than 2 %, add GEX Buffer as 5 volumes of the gel slice (100mg = 0.1ml).

3. **Incubate at 60℃ for 5-10 minutes until the gel is completely dissolved. Invert the tube every 1-2 minutes during incubation.**
   Ensure that the gel has been completely dissolved before proceeding to step 4. If the gel slice has not been completely dissolved, more GEX Buffer should be added.
   To increase DNA recovery rate, especially for fragment sizes <500bp and >4kb, add 0.25 volume of isopropanol to the mixture and mix well.

4. **Insert a GP™ Column into the luer-lock of a vacuum manifold (e.g. Promega's Vac-man). Load up to 0.7ml of the dissolved gel mixture onto the column.**

5. **Apply vacuum to draw all the liquid into the manifold. Repeat step 4 for the rest of the mixture.**

6. **Wash the column once with 0.5ml of WN Buffer by re-applying vacuum to draw all the liquid.**

7. **Wash the column once with 0.5ml of WS Buffer by re-applying vacuum to draw all the liquid.**

8. **Place the column onto a Collection Tube. Centrifuge the column at full speed for 3-5 minutes to remove residual ethanol.**
   It is important to remove residual ethanol, since it may inhibit subsequent enzymatic reactions.

9. **Place the column onto a new 1.5-ml centrifuge tube. Add 15-30µl of Elution Buffer onto the center of the membrane.**
   For effective elution, make sure that the elution solution is dispensed onto the center of the membrane and is completely absorbed.

10. **Stand the column for 2-3 minutes and centrifuge at full speed for 1-2 minutes to elute DNA.**

11. **Short-term (up to a few weeks), store at 4°C; long-term, store at -20°C. To avoid multiple freeze-thaw cycles, preparation of aliquots is recommended.**