metabion international AG
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Version: 01

mi-PCR Purification Kit

Cat. No. mi-PCR50 & mi-PCR250

Purification of small-scale DNA using phenol/chloroform extraction or ethanol precipitation is laborious and time-consuming. ***metabion***'s mi-PCR Purification Kit provides a simple and fast method to extract and isolate DNA fragments after enzymatic reactions from enzymes, dNTPs, salts and primers 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 60-95% 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-PCR50**	**mi-PCR250**
Preps	**50**	**250**
PX Buffer	30ml	150ml
WN Buffer	6ml	30ml
WS Buffer	6ml	30ml
Elution Buffer	5ml	25ml
GP Column	50	250
Collection Tube	50	250
Protocol	1	1

Storage Conditions

metabion's mi-PCR Purification 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.

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 13,000 rpm in a microcentrifuge, unless noted otherwise.
- For long-term storage of the eluted plasmid, 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-PCR50

- 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-PCR250

- 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. PCR Fragment Purification by Centrifugation

1. **Pipet 10-100µl PCR product (make sure that mineral oil is not taken) or DNA solution after enzymatic reaction to a new 1.5-ml centrifuge tube. Add 0.5ml PX Buffer and mix well.**
   To increase DNA recovery rate, especially for fragment sizes <500bp and >5kb, add 0.25 volume of isopropanol to the mixture and mix well.

2. **Place a GP Column onto a Collection Tube. Add the mixture from step 1 onto the column.**

3. **Centrifuge for 30-60 seconds. Discard the flow-through.**

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

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

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

7. **Place the column onto a new 1.5-ml centrifuge tube. Add 15-30µl of Elution Buffer (provided) 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.

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

9. **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. PCR Fragment Purification by Vacuum

1. **Pipet 10-100 µl PCR product (make sure that mineral oil is not taken) or DNA solution after enzymatic reaction to a new 1.5-ml centrifuge tube. Add 0.5ml PX Buffer and mix well.**
   To increase DNA recovery rate, especially for fragment sizes <500bp and >5kb, add 0.25 volume of isopropanol to the mixture and mix well.

2. **Insert a GP Column into the luer-lock of a vacuum manifold (e.g., Promega's Vac-man). Add the complete mixture from step 1 onto the column.**

3. **Apply vacuum to draw all the liquid into the manifold.**

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

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

6. **Place the column onto a Collection Tube. Centrifuge the column at full speed for another 3-5 minutes to remove residual ethanol.**
   It is important to remove residual ethanol, since it may inhibit subsequent enzymatic reactions.
7. **Place the column onto a new 1.5-ml centrifuge tube. Add 15-30µl of Elution Buffer (provided) 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.

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

9. **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.**