

(01.10.2009)

mi-PCR & Gel Duo-Purification Kit

Cat. No mi-PGD200
[200 Preparations]

**This kit is for research purposes only.
Not for use in diagnostic procedures.
For *in vitro* use only.**

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Introduction

mi-PCR & Gel Duo-Purification Kit is designed for quick and convenient procedure of PCR purification & Gel Extraction in less than 20 minutes. DNA Purification solution is added to the PCR product and the mixture is applied to a Spin Column containing glass fiber-based membranes where DNA is absorbed.

Also, Gel extraction mixture containing DNA products is applied to a glass fiber-based membrane in Spin Column. DNA polymerases, buffer, unreacted primers, dNTP and other contaminants are removed with ethanol-based Column Washing Solution. Since the DNA is eluted with nuclease-free water or Elution buffer (Tris-HCl, pH 8.0), precipitation is not necessary. This kit eliminates the use of organic solvent such as phenol and chloroform. The recovery of isolated DNA fragments which are between 100 bp and 10 kb in length can be obtained at least 80 %, and used directly for sequencing, cloning, and any kinds of enzymatic reactions.

Kit Contents

mi-Gel Extraction Kit

Preparation	200rxn /kit
DNA Purification solution	110 ml
Column Washing Solution	250 ml
Elution Buffer	15 ml
Spin Column	200
Collection Tube	200

Required Equipment

Microcentrifuge (13.000 rpm or 12.000 x g)
Vortexer
Microcentrifuge tubes
Water bath or heating block (65°C)
Distilled water (pH 7-8) or 10 mM Tris-HCL (pH 8.0)

Storage Conditions

mi-PCR & Gel Duo-Purification Kit should be stored at room temperature. Upon storage, especially at low temperature, a white precipitate may form in DNA purification Solution. Dissolve such precipitates by incubation of the bottle at 50°C before use.

Kit Storage

Room temperature for 6 months.

Precautions

See MSDS on our homepage.

Wear gloves and goggles. Avoid contact with all reagents. If eye or skin contact occurs, wash thoroughly with water. Avoid direct contact of DNA Binding Buffer with bleach or other oxidizers. **WARNING:** The Column Wash Buffer is flammable.

Protocol

Protocol for PCR Purification

1. Add 5 volumes of DNA Purification Solution to your PCR reaction and mix well. For example, add 500 ml of DNA Purification solution to a 100 ml of PCR reaction. It is not necessary to remove mineral oil.
2. Transfer the DNA/ DNA Purification Solution mixtures into a Spin Column with Collection Tube and centrifuge for 1 min. Remove the Spin Column and discard the liquid flow-through from the Collection Tube by decanting. And replace the Spin Column in the same decanted Collection Tube.
3. Add 600 ml of Column Washing Solution and centrifuge for 1 min. discard the flow-through. If the DNA will be used for salt sensitive applications, repeat the washing step or let the Spin Column for 5 min. at room temperature after adding Column Washing Solution.
4. Repeat the <Step 3> with 500 ml Column Wash Solution.
5. Centrifuge for an additional 1 min to remove residual Washing Solution. If residual Washing Solution is not completely removed, subsequent enzymatic reactions may be inhibited.
6. Place the Spin Column in a clean 1.5 ml micro centrifuge tube. Apply 20 to 100 ml of deionized water or 10 mM Tris-HCl, pH 8.0 directly in the middle of Spin Column membrane. Let it stand for 1min, and centrifuge for 1min.

D.W or 10 mM Tris-HCl (pH 8.0) passes through the Spin Column. The DNA is released (eluted) off the filter under the low-salt condition. Elution volume can be decreased to 20 ml minimum for high concentration or increased to 100 ml maximum for high efficiency of DNA recovery. If released DNA is too dilute on your purposes, concentrate by 0.1 volumes of 3 M Sodium Acetate and 2.5 volumes of 100% cold ethanol or isopropanol.

Protocol for Gel Extraction

1. Cut desired DNA band with a clean, sharp scalpel.
2. Add 3 volumes of DNA Purification Solution to the gel slice (v/w).
For example 100 mg of the gel slice requires 300 ml of Gel purification Solution. Be sure gel is submerged in the DNA purification Solution. Color of DNA purification Solution is yellow. > 2% agarose gels require 5 volumes of Gel Extraction Solution.
3. Incubate for 5~10 minutes at 50°C. Invert the tube every 2~3 min.
Mix thoroughly for 10 seconds, but do not vortex. Check that the slice has dissolved completely. If the color of mixture becomes purple after melting of the slice, add 10 ml of 3M sodium acetate (pH 5.0). The color will be turned to yellow. If the loading dye is included, the color will be changed to purple.
4. Transfer the of DNA Purification solution mixtures into a Spin Column with a Collection Tube and centrifuge for 1 min.
Remove the Spin Column and discard the liquid flow-through from the Collection Tube by decanting. And replace the Spin Column in the same decanted Collection Tube.
5. Add 600 ml of Column Washing Solution and centrifuge for 1 min. discard the flow-through.
If the DNA will be used for salt sensitive applications, repeat the washing step or let the Spin Column for 5 min. at room temperature after adding Washing Solution.
6. Repeat the <Step 5> with 500 µl of Column Washing Solution.
7. Centrifuge for an additional 1 min to remove residual Washing Solution.
If residual Washing Solution is not completely removed, subsequent enzymatic reactions may be inhibited.
8. Place the Spin Column in a clean 1.5 ml micro centrifuge tube. Apply 20 to 100 ml of deionized water or 10 mM Tris-HCl, pH 8.0 (Elution Buffer) directly in the middle of Spin Column membrane. Let it stand for 1min, and centrifuge for 1min.
D.W or 10 mM Tris-HCl (pH 8.0) passes through the Spin Column. The DNA is released (eluted) off the filter under the low-salt condition. Elution volume can be decreased to 20 ml minimum for high concentration or increased to 100 ml maximum for high efficiency of DNA recovery. If released DNA is too dilute on your purposes, concentrate by 0.1 volumes of 3 M Sodium Acetate and 2.5 volumes of 100% cold ethanol or isopropanol

Hints and Troubleshooting

Symptoms	Possible Causes	Comments
No or poor DNA yield	Incompletely solubilized Gel slice	Minimize gel volume by cutting the gel slice as small as possible. After addition of DNA purification Solution to the gel slice, mix every 2-3min.
	Incorrectly dispense of Elution buffer.	Elution buffer should be dispensed to center of membrane. Increase the elution volume to 200 μ l maximum for high efficiency of DNA recovery.
	Incorrectly dispense of Elution buffer	Elution buffer should be dispensed to center of membrane. Increase the elution volume to 200 μ l maximum for high efficiency of DNA recovery.
	Unsuitable Elution buffer	Elution efficiency is dependent on pH and salt concentration. The optimal efficiency is obtained pH 7.0~8.5 and in the presence of low-salt buffer (ex. 10 mM Tris-HCl, pH 8.5 or D.W). To ensure yield, preheat elution buffer to 70°C before elution. Apply Tris buffer or D.W directly onto the center of membrane.
Poor enzymatic reaction	Too high Salt concentration in eluate	The common choice is repetition of wash step. Alternatively, leave the spin column for 5 min at room temperature after adding Washing Solution.
	Residual Washing Solution in eluate	Centrifuge for an additional 1 min to remove residual Washing Solution.
	Smaller smeared band is appears on analytic gel.	In this case, denatured ssDNA can be contained in eluate. To reanneal the ssDNA, heat the eluate at 95°C for 2 min and allow the tube slowly at room temperature.
Degraded DNA	Sample is too old or mis-stored.	Old and mis-stored samples lead to degrade DNA.

If you have any questions or comments, please contact us at:

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