Quick PCR Purification and Gel Extraction Kit

Catalog Code: K004

Storage: Room Temperature

For PCR Purification and Gel Extraction

This package insert must be read in its entirety before using this product.

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Kit Contents

Size	50tests	200 tests
Buffer PB(for PCR Purification)	15 ml	60 ml
Buffer QG(for Gel Extraction)	30 ml	120 ml
Buffer PE	15 ml	60 ml
Spin Column CM	50	200

Storage

Fine quick Kits should be stored dry at room temperature (15-25℃). Under these conditions, Fine quick Kits can be stored for up to 12 months without showing any reduction in performance and quality. Check buffers for precipitate before use and redissolve at 37°C if necessary. The entire kit can be stored at 2–8°C, but in this case the buffers should be redissolved before use. Make sure that all buffers and spin colums are at room temperature when used.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

Buffer PB contains guanidine hydrochloride, which can form highly reactive compounds when combined with bleach. In case liquid containing this buffer is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1 % (v/v) sodium hypochlorite.

Introduction

Fine quick Kits is designed to extract high-quality DNA of 70 bp to 10 kb from PCR reactions, standard or low-melt agarose gels for molecular biology experiments. Up to 400 mg agarose gel can be processed per spin column.

Principle

The Fine quick system combines the convenience of spin-column technology with the selective binding properties of a uniquely designed silica membrane. Special buffers with the kit are optimized for efficient recovery of DNA and removal of contaminants in each specific application. DNA

adsorbs to the silica membrane in the presence of high concentrations of salt, while contaminants pass through the column. Impurities are efficiently washed away, and pure DNA is eluted with TE buffer or water.

Preparation

1. Add ethyl alcohol to PE Buffer bottle to make the ethanol final concentration 80% v/v.
2. 1.5ml centrifuge tube
3. Isopropanol

Procedure

1. For PCR purification , Add 5 volumes of Buffer PB to 1 volume of the PCR sample and mix. For example, add 500 µl of Buffer PB to 100 µl PCR samples (not including oil). Then to Step 3.
2. For gel extraction , Excise the DNA fragment from the agarose gel with a clean, sharp scalpel.Weigh the gel slice in a colorless tube. Add 3 volumes of Buffer QG to 1 volume of gel (100 mg ~ 100 µl). For >2% agarose gels, add 6 volumes of Buffer QG. The maximum amount of gel slice per column is 400 mg.
   Incubate at 50°C for 1 0 min (or until the gel slice has completely dissolved). To help dissolve gel, mix by vortexing the tube every 2–3 min during the incubation.
   IMPORTANT: Solubilize agarose completely. For >2% gels, increase incubation time.
   After the gel slice has dissolved completely, add 1 gel volume of isopropanol to the sample and mix.Then to Step 3.
3. Place a spin column in a provided 2 ml collection tube.
4. To bind DNA, apply the sample to the column and centrifuge for 30–60 s.
5. Discard flow-through. Place the column back into the same tube.
   For gel extraction, recommended: Add 0.5 ml of Buffer QG to column and centrifuge for 1 min.This step will remove all traces of agarose. It is only required when the DNA will subsequently be used for direct sequencing, in vitro transcription, or microinjection.
6. To wash, add 0.75 ml Buffer PE to the column and centrifuge for 30–60 s.
7. Discard flow-through and place the column back in the same tube. Centrifuge the column for an additional 1 min.
   IMPORTANT: Residual ethanol from Buffer PE will not be completely removed unless the flow-through is discarded before this additional centrifugation.
8. Place the column in a clean 1.5 ml microcentrifuge tube.
9. 9. To elute DNA, add 50 µl Buffer EB (1 0 mM Tris·Cl, pH 8.5) or water (pH 7.0–8.5) to the center of the column membrane , let the column stand for 1 min, and then centrifuge the column for 1 min.
   IMPORTANT: Ensure that the elution buffer is dispensed directly onto the column membrane for complete elution of bound DNA. Elution efficiency is dependent on pH. The maximum elution efficiency is achieved between pH 7.0 and 8.5. When using water, make sure that the pH

value is within this range, and store DNA at –20°C as DNA may degrade in the absence of a buffering agent. The purified DNA can also be eluted in TE buffer (1 0 mM Tris·Cl, 1 mM EDTA, pH 8.0), but the EDTA may inhibit subsequent enzymatic reactions.

Extraction Example

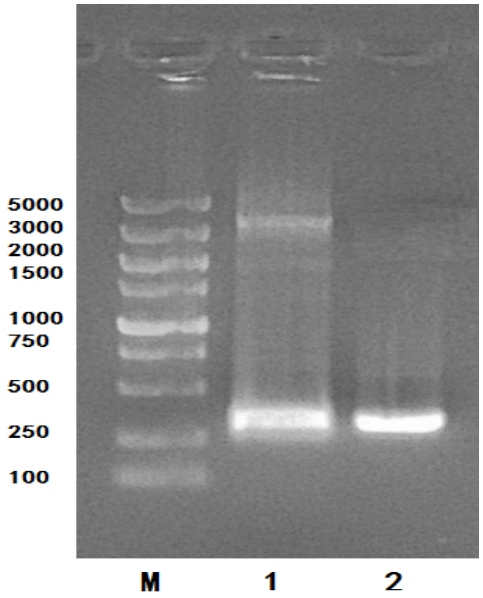

Fig. 1 Example: PCR production extracted by using **FineTest Kit**.

Lane M: DNA Marker

Lane 1: PCR production before gel recovery.

Lane 2: PCR production after gel recovery.

Troubleshooting Guide

Problem	Cause	suggestions
Low or no recovery	Buffer PE did not contain ethanol	Ethanol must be added to Buffer PE before use. Repeat procedure with correctly prepared Buffer PE.
	Inappropriate elution buffer	DNA will only be eluted efficiently in the presence of low-salt buffer (Buffer EB: 10 mM Tris·Cl, pH 8.5) or water.
	Elution buffer incorrectly dispensed	Add elution buffer to the center of the column membrane to ensure that the buffer completely covers the membrane.This is particularly important when using small elution volumes.
	Insufficient/no PCR product(for PCR purification)	Estimate DNA recovery by running10% of PCR product before and after purification on an agarose gel.
	Gel slice incompletely solubilized (for gel extraction)	After addition of Buffer QG to the gel slice, mix by vortexing the tube every 2–3 min during the 50°C incubation. DNA will remain in any undissolved agarose.
	pH of electrophoresis buffer too high(for gel extraction)	The electrophoresis buffer has been repeatedly used or incorrectly prepared, resulting in a sample pH that exceeds the buffering capacity of Buffer QG and leads to inefficient DNA binding. Add 10µl of 3 M sodium acetate, pH 5.0, to the sample and mix.
	Gel slice was too large (>400 mg) (for gel extraction)	70–80% recovery can only be obtained from ≤ 400 mg gel slice per column.
DNA does not perform well (e.g., in ligation reactions)	Salt concentration in eluate too high	Modify the wash step by incubating the column for 5 min at room temperature after adding 750 µl Buffer PE, then centrifuge.

Eluate contains residual ethanol	Ensure that the wash flow-through is drained from the collection tube and that the column is then centrifuged at 1 3,000 rpm for an additional 1 min.
Eluate contaminated with agarose (for gel extraction)	The gel slice is incompletely solubilized or weighs >400 mg. Repeat procedure, including the optional Buffer QG column-wash step.
Eluate contains primer dimers	Primer-dimers formed are >20 bp and are not completely removed. After the binding step, wash the column with 750 µl of a 35% guanidine hydrochloride aqueous solution (35 g in 1 00 ml).Continue with the Buffer PE wash step and the elution step as in the protocol.
Eluate contains denatured ssDNA , which appears as smaller smeared band on an ana-lytical gel	Use the eluted DNA to prepare the subsequent enzymatic reaction but omit the enzyme. To reanneal the ssDNA, incubate the reaction mixture at 95°C for 2 min, and allow the tube to cool slowly to room temperature. Add the enzyme and proceed as usual. Alternatively, the DNA can be eluted in 10 mM Tris buffer containing 10 mM NaCl. The salt and buffering agent promote the renaturation of DNA strands. However, the salt concentration of the eluate must then be considered for subsequent applications.