LaboPass ™ PCR

Introduction

LaboPass ™ PCR kit provides a simple and rapid method to purify PCR products or other enzymatic reactions in just 6 minutes. Up to 10 μg of pure DNA which is at least 100 bp but less than 50 kb in length can be obtained using this kit and the purified DNA can be directly used for sequencing, cloning and other routine applications without further manipulation. LaboPass ™ PCR procedures remove the DNA fragments smaller than 100 bp from the preparation, resulting in removal of primers or primer dimers of PCR.

Principle of methods

In the presence of high salt, DNA binds selectively to a glassfiber membrane in a LaboPass ™ spin column. The binding reaction occurs due to the disruption of the organized structure of water molecules and the interaction with the nucleic acid. The DNA remains bound while a series of rapid "wash-and-spin" steps removes contaminating small molecules (primers, nucleotide and salts). Finally, a low salt buffer from the glassfiber membrane elutes the purified DNA. The process does not require DNA precipitation, organic solvent extractions, or further handling.

Storage and Stability

LaboPass ™ PCR kit is shipped at room temperature. All solutions should be stored at 15 - 25℃. The kit components are guaranteed to be stable for 12 months from the date of manufacture printed on the product package. Please note that improper storage at 4℃ (refrigerator) or -20℃ (freezer) will adversely impact DNA purification when precipitates form in the buffers. The Buffer PB may exhibit salt precipitation due to cold temperatures as shipping or lab ambient conditions in winter. The procedure will not be performed optimally if the salt precipitates out of solution. If that happen in any of the solutions, warm the bottle at 50℃ with occasional mixing until completely dissolved.

Chemical Hazard

Buffer PB contains irritant which is harmful when in contact with skin or eyes, or when inhaled or swallowed. Care should be taken during handling. Always wear gloves and eye protector, and follow standard safety precautions.

Quality Control

All components in this kit are manufactured in particle-controlled area (class 1000 clean room), and its degree of cleanness is monitored periodically.

Kit Contents

Cat. No.	CMR0111	CMR0112	CMR0115
No. of preparation	50	200	1000
Spin column	50	200	1000
Collection tube	50	200	1000
Buffer PB	30ml	125ml	125mlx5
Buffer NW(conc.)	15ml	57ml	57mlx5
Buffer EB	15ml	15ml	15mlx5

* All Solution (Buffer) is available to purchase separately.

LaboPass ™ PCR kit Protocols

Before experiment

- All centrifugation should be carried out at 10,000 xg above (>12,000 rpm) at room temperature in a microcentrifuge.
- **Add absolute ethanol to Buffer NW as printed on the label before first use.**
- All solutions should be equilibrated at room temperature before procedures.

1. Add 5 volumes of Buffer PB to 1 volume of the sample and mix. Transfer the mixture to a Spin Column.

For 100 ㎕ reaction, add 500 ㎕ of Buffer PB. It is not necessary to remove mineral oil. If precipitated material has formed in buffer PB, heat to dissolve at 37℃.

2. Centrifuge for 1 min. Discard the flow through and reinsert the Spin Column back into the same tube.

3. Add 750 ㎕ of Buffer NW. Centrifuge for 1 min at full speed. Transfer the Spin Column into a new 1.5 ml tube.

If the Spin Column has Buffer NW associated with it, centrifuge again for additional 1 min at full speed before transferring to a new 1.5 ml tube. Residual ethanol from buffer NW may inhibit subsequent enzymatic reaction.

4. Apply 50 ㎕ of Buffer EB or dH_2O to the center of the membrane in the Spin Column, let stand for 1 min, and centrifuge for 1 min.

Elution volume can be reduced to 30 ㎕ for higher concentration of elute. Ensure that the buffer EB or distilled water is dispensed directly onto the center of spin column membrane for optimal elution of DNA. For long-term storage, eluting in buffer EB (10mM Tris-Cl. pH 8.5) or TE, pH 8.0 and storing at –20℃ is recommended. When using water for elution, check the pH of water (>pH7.0) before elution.

Troubleshooting guide

Low or no recovery

Buffer NW prepared incorrectly

- Ensure that the appropriate volume of absolute ethanol printed on the bottle label is added to Buffer NW.

Improper elution buffer

- As user's requirement, elution buffer other than Buffer EB can be used. However, the conditions of optimal elution should be low salt concentration with alkaline pH (7<pH<9). When water or other buffer was used as eluent, ensure that conditions.

Elution buffer incorrectly dispensed

- Ensure that elution buffer dispensed to the center of membrane. Incorrectly dispensed elution buffer causes inappropriate contact with membrane, followed by poor DNA recovery.

Enzymatic reactions is not performed well with purified DNA

Residual ethanol from Buffer NW remains in eluate

- Discard flow-through from step 3, and then centrifuge for additional 2 min to remove ethanol completely before proceed to step 4.

Eluate contains denatured ddDNA

- For reannealing of ssDNA to dsDNA, incubate ssDNA at 95℃ for 2 min, and then allow to cool to room temperature.

DNA floats out of well while loading of agarose gel

Residual ethanol from Buffer NW remains in eluate

- Discard flow-through from step 3, and then centrifuge for additional 2 min to remove ethanol completely before proceed to step 4.