LaboPass ™

Gel

Protocol Book

LaboPass ™ GEL

Introduction

LaboPass ™ Gel kit is designed for a fast and efficient isolation of 100 bp to 10 kb of DNA fragments from TAE or TBE agarose gel. This kit can extract up to 10 µg of pure DNA fragments from standard agarose gel as well as low melting agarose gel. DNA fragments isolated with this kit can be directly applied in ligation, labeling, or most of enzymatic reactions without further manipulation.

Principle of methods

In the presence of high salt, the product DNA binds selectively to a glass fiber 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, nucleotides and salts). Finally, a low salt buffer elutes the DNA from the glass fiber membrane. The process does not require alcohol precipitation, organic solvent extraction, or any further handling.

Storage and Stability

LaboPass ™ Gel 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 GB may exhibit salt precipitation due to cold temperatures as shipping or lab ambient conditions in winter. The product will not perform optimally if the salt precipitates out of solution. If that happen in any of the solutions, warm the bottles at 50℃ with occasional mixing until completely redissolved.

Chemical Hazard

Buffer GB 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

DNA molecular weight marker is separated on a 1 % agarose gel and fragments of various size are isolated according to the kit protocol. The recovery of the isolated DNA fragments was at least 80 %. Isolated fragments are readily digested with the restriction enzyme showing expected pattern. 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.	CMG0111	CMG0112	CMG0115
No. of preparation	50	200	1000
Spin column	50	200	1000
Collection tube	50	200	1000
Buffer GB	30ml	125ml	125mlx5
Buffer NW(conc.)	15ml	57ml	57mlx5
Buffer EB	15ml	15ml	15mlx5

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

LaboPass ™ GEL 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. Excise the DNA band of interest using an ethanol-cleaned razor blade or scalpel on a transilluminator.

Minimize gel volume by cutting the gel slice as small as possible.

2. Weigh the gel slice in a microcentrifuge tube. Add 3 volumes of Buffer GB to 1 volume of gel (100mg).

For example, add 300 µl of Buffer GB to each 100 mg of agarose gel slice. For >1.5 % agarose gel, add 5 volumes of Buffer GB. If precipitated material has formed in buffer GB, heat to dissolve at 37℃.

3. Incubate at 50℃ until the agarose gel is completely melted (5-10 minute). To help dissolve gel, mix by vortexing the tube every 2-3 minutes during the incubation.

4. After the slice has dissolved completely, check that the color of the mixture is yellow. (similar to Buffer GB)

If the color of the mixture is dark orange or purple, add 10 µl of 3 M sodium acetate, pH 5.0, and mix. The color of the mixture will turn yellow.

5. (Optional) Add 1 gel volume of isopropanol to the sample and mix. Transfer the mixture to a Spin Column.

This step increases the yield of DNA fragments < 500 bp and >4 kb. For DNA fragments between 500 bp and 4 kb, addition of isopropanol has no effect on yield.

6. Centrifuge for 1 min. Remove the Spin Column from the tube and discard the flow through from the Collection Tube. Reinsert the Spin Column into the Collection Tube.

7. Add 750µl of Buffer NW to the Spin Column. Let it stand for 5 minutes, making some amount of wash solution flow through the column.

8. Centrifuge for 1 min. Transfer the Spin Column to a new, sterile 1.5 ml tube.

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

9. Apply 50µl 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.

Ensure that the Buffer EB or distilled water is dispensed directly onto the center of spin column membrane for optimal elution of DNA. To obtain more concentrated DNA solution, add 30 µl of Buffer EB or dH_2O. 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.

Gel slice incompletely solubilized

- After addition of Buffer GB to the gel slice, mix by vortexing the tube every 2–3 minutes during the 50℃ incubation. DNA will remain in any undissolved agarose.

Binding mixture turns orange or violet

- The pH in the sample exceeds the buffer capacity of Buffer GB. Add 10 µl of 3 M sodium acetate, pH 5.0, to the sample and mix. The color of the mixture will turn yellow indicating the correct pH for DNA binding. Even for binding mixtures with only small color changes (slight orange color), add the 10µl sodium acetate.

Cloudy and **gelatinous** appearance of sample mixture after addition of isopropanol

-This may be due to salt precipitation, and will disappear upon mixing the sample.

Enzymatic reactions is not performed well with purified DNA

Salt concentration in eluate too high

- Modify the wash step by incubating the spin column for 5 min at room temperature after adding 750 µl of Buffer PE, then centrifuge

DNA floats out of well while loading of agarose gel

Residual ethanol from Buffer NW remains in eluate
- Discard flow-through from step 8, and then centrifuge for additional 2 min to remove ethanol completely before proceed to step 9.

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