***Ver: 2401***

**DNA Gel/PCR Purification Maxprep Kit**

**（BW-DC3531）**

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# Kit Contents

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| --- | --- | --- | --- |
| Catalog# | BW-DC3531-00 | BW-DC3531-01 | BW-DC3531-02 |
| Preps | 10 | 50 | 100 |
| ezBindTM Maxi Columns | 2 | 10 | 25 |
| 50mL Collection Tube | 2 | 10 | 25 |
| Buffer GC | 100mL | 500 mL | 1250 mL |
| DNA Wash Buffer\* | 15 mL | 54 mL | 2×54 mL |
| Elution Buffer | 6 mL | 30 mL | 60 mL |
| User Manual | 1 | 1 | 1 |

\*Add 60 mL (DC3531-00) or 216 mL (DC3531-01) or 216 mL (DC3531-02) 96-100% ethanol to each DNA Wash Buffer bottle before use.

Buffer GC may form precipitates under cool ambient condition. Warm up the buffer at 37°C to dissolve before use.

# Introduction

This fast and reliable kit is designed to recover DNA from agarose gels and purify DNA fragments from PCR, RFLP, phosphorylation, labeling, ligation, hybridization, and other enzymatic reactions. DNA fragments from 100 bp to 20 kb can be purified using the Mini Column with over 50-90% recovery.

# Storage and Stability

All components can be stored at room temperature (15-25℃). All kit components are guaranteed for 12 months from the date of production.

# Before Starting

Prepare all components and get all necessary materials ready by examining this user manual and become familiar with each step.

# Important Notes

* A gel slice of 1000 mg equals to approximately 1000 µL.
* Buffer GC may form precipitates under cool ambient condition. Warm up the buffer at 37℃ to dissolve before use.
* Buffer GC contains an integrated pH indicator, allowing easy determination of the optimal pH for DNA binding. DNA adsorption requires pH ≤7.5, and the pH indicator in the buffers will appear yellow in this range. If the pH is >7.5, and the pH indicator in the buffers will appear pink in this range. This means that the pH of the sample exceeds the buffering capacity of Buffer GC, and DNA adsorption will be inefficient. In these cases, the pH of the binding mixture can easily be corrected by adding 10µL 3 M sodium acetate\*, pH 5.0 to per sample, before proceeding with the protocol.
* Preheat aliquots of Elution Buffer or ddH2O at 65℃ water bath.

# Materials not Supplied

❂Tabletop microcentrifuge and 50 mL tubes.

❂55-65℃ water bath.

❂Vacuum manifold if use vacuum protocol.

❂96~100% ethanol.

❂Isopropanol for DNA fragment less than 200 bp.

*Perform all steps including centrifugation at room temperature!*

# Safety Information

Buffer GC contains acidic acid and chaotropic salts, which may form reactive compounds when combines with bleach. Do not add bleach or acidic solutions directly to the waste.

# Protocol (For spin)

1. **For cycle-pure (PCR reaction):** Add **2 volumes** of **Buffer GC** to **1 volume** of the **PCR reaction** and mix completely by vortex. Briefly spin the tube to collect any drops from the inside wall and tube lid.

**Note:** PCR products less than 200 bp, add 5 volumes of Buffer GC to 1 volume of PCR reaction. **Note:** For DNA fragment less than 200 bp more than 4000bp, add 1 volume of isopropanol.

**For** **agarose gel:** Excise the DNA fragment from the agarose gel and weigh it in a 15 mL tube. Add **1 volume** of **Buffer GC** to **1 volume** of **gel** to the 15 mL tube and incubate the mixture at 55-60℃ for 8-10 min. Mix the tube by tapping the bottom every 2 min till the gel has melted completely. Cool the tube to room temperature.

**Note:** A gel slice of 1000 mg approximately equals to 1000 µL.

**Note:** For >2% gel, add 2 volume GC.

**Note:** The maximum amount of gel slice per Mini column is 400 mg; for gel slices >400 mg, use more than one Mini column.

**Note:** For DNA fragment less than 200 bp/ more than 4000bp, add 1 volume of isopropanol.

1. Transfer up to **DNA/Buffer GC mixture** to a **Max Column** with a **50 mL Collection Tube**. Centrifuge at 12,000 rpm for 1 min at room temperature. Discard the flow-through and put the column back to the **50 mL Collection Tube**. Repeat this step to process the remaining sample.
2. Add **5mL** **DNA Wash Buffer** to the column and centrifuge at 12,000 rpm for 1 min. Discard the flow through and insert the column, with the lid open, back to the collection tube.

**Note:** Ensure that ethanol has been added to DNA Wash Buffer as instructed.

1. Repeat step **3**.
2. **Optional：**For agarose gel: Add **5mL** 100% ethanolto the column and centrifuge at 12,000 rpm for 30 s. Discard the flow through and insert the column, with the lid open, back to the collection tube.

**Note:** If the DNA will be used for salt-sensitive applications (e.g., sequencing, blunt-ended ligation), this step is recommended.

1. Centrifuge the empty **Max Column**, with the lid open, at 12,000 rpm for 2 min to dry the ethanol residue in the matrix.

**Note:** The residual ethanol will be removed more efficiently with the column lid open during centrifugation.

1. Place the column into a **50 mL Microfuge Tube** and add **1-1.5mL** preheated (65℃) **Elution Buffer** or ddH2O to the center of the column. Incubate at room temperature for 1 min. Centrifuge at 12,000 rpm for 1 min to elute the DNA.

**Optional:** Reload the eluted DNA solution to the column for a second elution.

**Note:** Preheat Elution Buffer or ddH2O at 65℃ and incubate the column at 65℃ for 5 min after adding Elution Buffer or ddH2O will increase the DNA yield.

**Note:** For fragment larger than 8 kb, incubate the column at 65℃ for 5 min after adding Elution Buffer or ddH2O before centrifugation.

**Note:** The first elution normally yields 60-70% of the DNA. Reload the eluted DNA solution to the column for a second elution will yield another 20% of the DNA.

# Trouble Shooting Guide

|  |  |  |
| --- | --- | --- |
| **Problems** | **Possible Reasons** | **Suggested Improvements** |
| Low DNA yield  | 1. Not enough Buffer GC.
2. Agarose gel doesn’t melt completely.
3. Reused electrophoresis buffer with increased pH.
4. Fragment < 200 bp.
5. Fragment >10 kb.
 | 1. Determine the volume of Buffer GC to be used correctly as instructed.
2. Make sure to set the water bath to 55-60℃ to allow gel to melt completely. Add more Buffer GC if necessary.
3. Use fresh electrophoresis buffer.
4. Add isopropanol as instructed.
5. Incubate the column (after adding ddH2O or Elution Buffer) at 65℃ for 15 min before elution.
 |
| No DNA yield | Forgot to add ethanol to DNA Wash Buffer. | Add absolute ethanol to DNA Wash Buffer as instructed before use.  |
| DNA sample floats out of well while loading agarose gel | Ethanol was not completely removed from the column following wash step. | After the wash step, centrifuge the empty column with the lid open at top speed for 1-3 min. Repeat once.  |
| Column clogged | Agarose gel doesn’t melt completely. | Make sure to melt the gel at 55-60℃ before loading the sample to Mini Column. |

# Limited Use and Warranty

This product is intended for *in vitro* research use only. Not for use in human.

This product is warranted to perform as described in its labeling and in BEIWO’s literature when used in accordance with instructions. No other warranties of any kind expressed or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by BEIWO. BEIWO’s sole obligation and purchaser’s exclusive remedy for breach of this warranty shall be, at the option of BEIWO, to replace the products, BEIWO shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product.

For technical support or learn more product information, please contact us or visit our website.



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