

## **BW-DC3718 MV Gel Extraction Kit**

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## Kit Contents

Catalog#	BW-DC3718-00	BW-DC3718-01	BW-DC3718-02	BW-DC3718-03
Preps	10	50	100	250
Micro Columns	10	50	100	250
2 mL Collection Tubes	10	50	100	250
1.5 mL Microfuge Tubes	10	50	100	250
Buffer GC	5 mL	25 mL	50 mL	120 mL
DNA Wash Buffer*	3 mL	15 mL	2×15 mL	3×24 mL
Elution Buffer	1 mL	5 mL	5 mL	10 mL
User Manual	1	1	1	1

\*Add 12 mL (BW-DC3718-00) or 60 mL (BW-DC3718-01) or 60 mL (BW-DC3718-02) or 96 mL (BW-DC3718-03) 96-100% ethanol to each DNA Wash Buffer bottle before use.

## Introduction

This fast and reliable kit is designed to recover up to 5 µg DNA from agarose gels. DNA fragments from 100 bp to 20 kb can be purified using the micro column with over 80-90 % recovery. Up to 400 mg agarose can be processed per Micro column.

## Storage and Stability

All components can be stored at room temperature (15-25°C). 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

- ☉ Add 96-100% ethanol to DNA Wash Buffer as Follows:

Add 12 mL (BW-DC3718-00) or 60 mL (BW-DC3718-01) or 60 mL (BW-DC3718-02) or 96 mL (BW-DC3718-03) 96-100% ethanol to each DNA Wash Buffer bottle before use.

- ☉ A gel slice of 100 mg equals to approximately 100  $\mu$ L.

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

- ☉ Preheat aliquots of Elution Buffer or ddH<sub>2</sub>O at 65°C water bath.

## Materials not Supplied

- ☉ Tabletop microcentrifuge and 1.5 mL microtubes.

- ☉ 55-65°C 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. Excise the DNA fragment from the agarose gel and weigh it in a 1.5 mL microtube. Add **1 volume** of **Buffer GC** to **1 volume** of **gel** to the 1.5 mL microtube and incubate the mixture at 55-60°C 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:** PCR products less than 200 bp, add 5 volumes of Buffer GC to 1 volume of PCR reaction.

**Note:** A gel slice of 100 mg approximately equals to 100 µL.

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

2. Transfer up to **700 µL DNA/Buffer GC mixture** to a **Micro Column** with a **2 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 **2 mL Collection Tube**. Repeat this step to process the remaining sample.

3. Add **500 µL DNA Wash Buffer** to 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:** Ensure that ethanol has been added to DNA Wash Buffer as instructed.

4. Repeat step **3**.

5. **Optional:** Add **600 µL** 100% ethanol to 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.

6. Centrifuge the empty **Micro 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.

7. Place the column into a **1.5 mL Microfuge Tube** and add **30 µL** preheated (65°C) **Elution Buffer** or ddH<sub>2</sub>O 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 ddH<sub>2</sub>O at 65°C and incubate the column at 65°C for 5 min after adding Elution Buffer or ddH<sub>2</sub>O will increase the DNA yield.

**Note:** For fragment larger than 8 kb, incubate the column at 65°C for 5 min after adding Elution Buffer or ddH<sub>2</sub>O 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.

### **Protocol (For vacuum)**

1. Follow the instruction described on step **1** on page **4**. Briefly spin the tube to collect any drops from the inside wall and tube lid.
2. Prepare the vacuum manifold according to manufacturer's instructions. Attach a **Micro Column** to the manifold.
3. Load the **DNA/Buffer GC mixture** to the **Micro Column** attached to the manifold. Turn on the vacuum to let the solution pass through the column.
4. Wash the column by adding **500 µL DNA Wash Buffer**. Vacuum the column for 1 min.
5. Repeat step **4**.
6. **Optional:** Add **600 µL** 100% ethanol to the column and Vacuum the column for 1 min.

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

7. Turn on the vacuum, dry the empty column for 5 min.
8. Put the column to a **1.5 mL Microfuge Tube** and add **30 µL Elution Buffer** or ddH<sub>2</sub>O to the column. Incubate at room temperature for 1 min. Centrifuge the tube at 12,000 rpm for 1 min to elute DNA.

**Note:** Preheat Elution Buffer or ddH<sub>2</sub>O at 65°C and incubate the column at 65°C for 5 min after adding Elution Buffer or ddH<sub>2</sub>O 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.

## Trouble Shooting Guide

Problems	Possible Reasons	Suggested Improvements
Low DNA yield	<ol style="list-style-type: none"> <li>1. Not enough Buffer GC.</li> <li>2. Agarose gel doesn't melt completely.</li> <li>3. Reused electrophoresis buffer with increased pH.</li> <li>4. Fragment &lt; 200 bp.</li> <li>5. Fragment &gt;10 kb.</li> </ol>	<ol style="list-style-type: none"> <li>1. Determine the volume of Buffer GC to be used correctly as instructed.</li> <li>2. Make sure to set the water bath to 55-60°C to allow gel to melt completely. Add more Buffer GC if necessary.</li> <li>3. Use fresh electrophoresis buffer.</li> <li>4. Add isopropanol as instructed.</li> <li>5. Incubate the column (after adding ddH<sub>2</sub>O or Elution Buffer) at 65°C for 15 min before elution.</li> </ol>
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°C 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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