***Ver: 1907***

**BW-GD2212 FFPE gDNA Isolation Kit (Column)**

**浙杭械备20200109**

**Contents**

[Kit Contents 2](#_Toc15288967)

[Introduction 2](#_Toc15288968)

[Storage and Stability 3](#_Toc15288969)

[Before Starting 3](#_Toc15288970)

[Important Notes 3](#_Toc15288971)

[Materials not Supplied 3](#_Toc15288972)

[Safety Information 3](#_Toc15288973)

[Simplified protocol without Proteinase K digestion 4](#_Toc15288974)

[Standard protocol with Proteinase K digestion 5](#_Toc15288975)

[Trouble Shooting Guide 7](#_Toc15288976)

[Limited Use and Warranty 8](#_Toc15288977)

# Kit Contents

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| **Catalog#** | **BW-GD2212-00** | **BW-GD2212-01** | **BW-GD2212-02** |
| Preps | 10 | 50 | 250 |
| DNA Micro Columns | 10 | 50 | 250 |
| 2 mL Collection Tubes | 10 | 50 | 250 |
| FFPE A | 1.2 mL | 6 mL | 30 mL |
| Buffer TL | 6 mL | 30 mL | 135 mL |
| Buffer BL | 2.4 mL | 12 mL | 60 mL |
| DNA Wash Buffer\* | 2 mL | 15 mL | 3 x 24 mL |
| Elution Buffer | 600 μL | 10 mL | 15 mL |
| L solution | 24 μL | 120 μL | 600 μL |
| Proteinase K | 240 μL | 1.2 mL | 6 mL |
| RNase A (20 mg/mL) | 110 μL | 550 μL | 2.7 mL |
| User Manual  | 1 | 1 | 1 |

\*Add 8 mL (BW-GD2212-00) or 60 mL (BW-GD2212-01) or 96 mL (BW-GD2212-02) 96-100% ethanol to each DNA Wash Buffer bottle before use.

# Introduction

The FFPE gDNA Isolation Kit is designed for genomic DNA purification from formalin-fixed, paraffin-embedded (FFPE) tissue samples. Instead of using xylene, the kit utilizes a proprietary nontoxic deparaffinization reagent for safe and convenient paraffin removal. To overcome the cross linking of nucleic acids caused by formalin fixation, specially formulated buffers and lysing conditions are developed to release DNA from tissue sections. The purified genomic DNA is suitable for downstream applications such as quantitative real-time RT-PCR, sequencing and mutation screening. Because tissue fixation and embedding cause significant fragmentation, DNA recovered from FFPE samples exhibits broad size distribution and is not recommended for downstream applications that require full length DNA.

# Storage and Stability

From the date of production, all components are stable for 12 months when stored accordingly. Store L solution at -20℃, Proteinase K at 4℃ upon receiving. Store all other components at room temperature (15-25℃).

# Before Starting

Prepare all components and get all necessary materials ready by examining this user manual and become familiar with each step and pay special attention to the followings.

# Important Notes

❂ Add 8 mL (BW-GD2212-00) or 60 mL (BW-GD2212-01) or 96 mL (BW-GD2212-02) 96-100% ethanol to each DNA Wash Buffer bottle before use.

❂ Add L solution to aliquot of Buffer BL at 1:100 (V:V) and store at 4℃. The Buffer BL/L solution mixture is stable for 1 month at 4℃.

# Materials not Supplied

❂ Sterile collection tubes.

❂ Sterile microfuge tubes.

❂ Water bath (98℃/55℃/65℃).

# Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets.

# Simplified protocol without Proteinase K digestion

1. Cut **3-5** paraffin sample sections between 5-10 μM thickness (up to 15 mg) and add the sample to a 1.5 mL microfuge tube.
2. Add **100 μL FFPE A** and **500 μL Buffer TL**, mix well by vortexing for 30 seconds.
3. Incubate at 98℃ for 30-60 minutes. Vortex several times during incubation. Cool down the sample to room temperature.

**Note:** Wear safety goggle and gloves. Use heat proof safe lock tubes to avoid liquid splash during incubation.

1. Spin the sample at 12,000 rpm for 5 minutes. Transfer the clear phase (middle layer) into a clear tube, avoid the impurities.
2. **Optional:** If RNA free gDNA is desired, add **10 μL RNase A (20 mg/ml)** and incubate at room temperature (15-25℃) for 2 minutes.
3. Add **200 μL Buffer BL** (*Add* ***L solution*** *to Buffer BL before use*)and **2 volumes** of 100% ethanol(for example, 1 mL ethanol for 500 μL Buffer BL and clear phase mixture), mix well by vortexing for 10 seconds.
4. Transfer the sample to a **DNA** **Micro Column** and spin at 12,000 rpm for 1 minute. Discard the flow-through liquid and reuse the collection tube.
5. Add **600 μL DNA Wash Buffer** (*Add ethanol to DNA Wash Buffer before use*) into the **DNA** **Micro Column**, centrifuge at 12,000 rpm for 1 minute, discard the flow-through.

**Optional:** Repeat step **8**.

1. Reinsert the spin column, with the lid open, into the collection tube and centrifuge for 2-5 minutes at 12,000 rpm.

**Note:** Residual ethanol can be removed more efficiently with the column lid open. It is critical to remove residual ethanol completely.

1. Carefully transfer the **DNA Micro Column** into a sterile 1.5 mL tube and add **30-50 μL (>30 μL)** sterile ddH2O or **Elution Buffer (preheating at 65℃)** into the center of the column and let it stand for 2-5 minutes. Elute the DNA by centrifugation at 12,000 rpm for 2 minutes. The DNA product should be stored at -20℃.

**Optional:** Reload the eluate into the center of the column for a second elution.

**Note:** The first elution normally yields around 70% of the plasmid DNA. Add the eluted DNA back to the column for another elution yields 20-30% of the DNA.

**Note:** The pH of the eluent has a great influence on the elution efficiency. If ddH2O is used as the eluent, the pH should be within the range of 7.0 to 8.5, and the DNA product should be stored at -20℃ to prevent its degradation.

# Standard protocol with Proteinase K digestion

1. Cut **3-5** paraffin sample sections between 5-10 μM thickness (up to 15 mg) and add the sample to a 1.5 mL tube.
2. Add **100 μL FFPE A** and **500 μL Buffer TL**, mix well by vortexing for 30 seconds.
3. Incubate at 98℃ for 30-60 minutes. Vortex several times during incubation. Cool down the sample to room temperature.

**Note:** Wear safety goggle and gloves. Use heat proof safe lock tubes to avoid liquid splash during incubation.

1. Spin the sample at 12,000 rpm for 5 minutes. Transfer the clear phase (middle layer) into a clear tube, avoid the impurities.
2. Add **20 μL Proteinase K** (vortex before use), mix well by pipetting, and incubate at 55℃ for 10 minutes.
3. **Optional:** If RNA free gDNA is desired, add **10 μL RNase A (20 mg/ml)** and incubate at room temperature (15-25℃) for 2 minutes.
4. Add **200 μL Buffer BL** (*Add* ***L solution*** *to Buffer BL before use*)and **2 volumes** of 100% ethanol, mix well by vortexing for 10 seconds.
5. Transfer the sample to a **DNA** **Micro Column** and spin at 12,000 rpm for 1 minute. Discard the flow-through liquid and reuse the collection tube.
6. Add **600 μL DNA Wash Buffer** (*Add ethanol to DNA Wash Buffer before use*) into the **DNA** **Micro Column**, centrifuge at 12,000 rpm for 1 minute, discard the flow-through.

**Optional:** Repeat step **9**.

1. Reinsert the spin column, with the lid open, into the collection tube and centrifuge for 2-5 minutes at 12,000 rpm.

**Note:** Residual ethanol can be removed more efficiently with the column lid open. It is critical to remove residual ethanol completely.

1. Carefully transfer the **DNA** **Micro Column** into a sterile 1.5 mL tube and add **30-50 μL (>30 μL)** sterile ddH2O or **Elution Buffer (preheating at 65℃)** into the center of the column and let it stand for 2-5 minutes. Elute the DNA by centrifugation at 12,000 rpm for 2 minutes. The DNA product should be stored at -20℃.

**Optional:** Reload the eluate into the center of the column for a second elution.

**Note:** The first elution normally yields around 70% of the plasmid DNA. Add the eluted DNA back to the column for another elution yields 20-30% of the DNA.

**Note:** The pH of the eluent has a great influence on the elution efficiency. If ddH2O is used as the eluent, the pH should be within the range of 7.0 to 8.5, and the DNA product should be stored at -20℃ to prevent its degradation.

# Trouble Shooting Guide

|  |  |  |
| --- | --- | --- |
| **Problems** | **Possible Reasons** | **Suggested Improvements** |
| Low DNA yields | Tissue was not digested. | Ensure that the tissue is fully submerged during the deparifinization step. |
| Insufficient lysis. | Proteinase K was stored at high temperatures for a prolonged time. Repeat the procedure using new samples and fresh Proteinase K. Make sure that the samples were thoroughly dehydrated prior to embedding. Residual formalin can inhibit the Proteinase K digest. |
| DNA Wash Buffer and/or may not be prepared correctly. | Prepare buffers accordingly. |
| Binding was incomplete. | Ensure that the samples are mixed well after adding Buffer BL and ethanol. Increase pipette mixing or vortexing to ensure complete mixing. |
| Problems in downstream applications | DNA is over fixated during tissue formalin fixation. | Extend incubation time at 90℃ to 90 minutes. |

# Limited Use and Warranty

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, express 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 at 400-115-2855 or visit our website at [www.biomiga.com.cn](http://www.biomiga.com.cn)