***Ver: 2311***

**BW-GD2211 Tissue gDNA Isolation Kit**

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# Kit Contents

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| --- | --- | --- | --- |
| **Catalog#** | **BW-GD2211 -00** | **BW-GD2211-01** | **BW-GD2211-02** |
| Preps | 4 | 50 | 250 |
| DNA Mini Columns | 4 | 50 | 250 |
| 2 mL Collection Tubes | 4 | 50 | 250 |
| Buffer TL | 1.0 mL | 13 mL | 65 mL |
| Buffer BL | 1.0 mL | 13 mL | 65 mL |
| Buffer KB | 3.0 mL | 26 mL | 130 mL |
| DNA Wash Buffer\* | 2.0 mL | 15 mL | 3 x 24 mL |
| Elution Buffer | 1.0 mL | 11 mL | 55 mL |
| Proteinase K | 110 μL | 1.3 mL | 5 x 1.3 mL |
| User Manual | 1 | 1 | 1 |

\* Add 8 mL (BW-GD2211-00) or 60 mL (BW-GD2211-01) or 96 mL (BW-GD2211-02) 96-100% ethanol to each DNA Wash Buffer bottle before use.

# Introduction

The Tissue gDNA Isolation Kit provides an easy and rapid method for the isolation of genomic DNA for consistent PCR and Southern analysis. Up to 30 mg of animal tissue, 5×106 culture cells, 0.2-0.5cm of mouse tail snips and 30 mg paraffin-embedded tissue （2-3 mm2） can be readily processed at a time. This kit allows for the single or multiple simultaneous processing of samples. There is no need for phenol/chloroform extractions, and time-consuming steps are eliminated (e.g. precipitation using isopropanol or ethanol). Purified DNA can be directly used for most applications such as PCR, Southern Blotting, and Restriction Enzyme Digestion. The binding capacity per column is 100 μg of gDNA. Use less than 30 mg of tissue is recommended.

# Storage and Stability

All Tissue gDNA Kit components are guaranteed for at least 12 months from the date of production when stored as follows:

Proteinase K is stable at room temperature (15-25℃) for one year. For long term, store aliquots at -20℃.

Store all other materials at room temperature (15-25℃).

# Before Starting

The kit is designed to be simple, fast, and reliable provided that all steps are followed diligently. Please read the entire booklet and get all necessary supplies and equipments.

# Important Notes

❂ Dilute DNA Wash Buffer with absolute ethanol as follows: Add 8 mL (BW-GD2211-00) or 60 mL (BW-GD2211-01) or 96 mL (BW-GD2211-02) of absolute ethanol to each bottle. The final concentration is 80%.

❂ Buffer BL and Buffer TL may form precipitates upon storage, dissolve the precipitates at 50℃ before use.

❂ Place the Elution Buffer in a 65℃ warter bath (0.5 mL per tube is recommended).

# Materials not Supplied

❂ Tabletop Centrifuge

❂ Sterile 1.5 mL centrifuge tubes

❂ Water bath

❂ Absolute ethanol

❂ Buffer PBS

# Safety Information

Buffer BL contains chaotropic salts, which may form reactive compounds when combines with bleach, Do not add bleach or acidic solutions directly to the preparation waste, wear gloves and protective eyewear when handling.

# A: Tissue gDNA Isolation Protocols

This method is suitable for the isolation of genomic DNA from up to 30 mg of tissue.

**Optional:** Although no mechanical homogenization of tissue is necessary, pulverizing the samples in liquid nitrogen will improve lysis and reduce incubation time. Once the liquid nitrogen has evaporated, transfer the powdered tissue into a clean 1.5 mL tube. Add **200 μL** of **Buffer TL** and proceed to step **2** below.

1. Mince up to **20-30 mg** tissueand place it into a1.5 mL centrifuge tube. Add **200 μL** **Buffer TL**. In order to speed up lysis, cut the tissue into small pieces.
2. Add **25 μL Proteinase K**. Vortex to mix, and incubate in a shaking water bath set to 50℃ to effectively complete lysis. If no shaking water-bath is available, vortex the sample every 20-30 minutes.

**Note：**lysis time depends on the amount and type of tissue used; average time is usually under 3 hours. One can allow lysis to proceed overnight.

1. **Optional:** Certain tissues such as liver tissue have high levels of RNA, which will be co-purified with DNA using this kit. While it will not interfere with PCR, the RNA may be removed at this point. To do so, add 4 μL (assuming a sample size of 30 mg) RNase A (20 mg/mL) and incubate at room temperature for 2 minutes. Proceed with the tissue protocol.
2. Centrifuge for 5 minutes at ≥12,000 ×g to pellet insoluble tissue debris. Carefully aspirate the supernatant and transfer to a sterile centrifuge tube.
3. Add **220 μL Buffer BL**. Vortex to mix. Incubate at 70℃ for 10 minutes.

**Note：**A wispy precipitate may form upon addition of Buffer BL, but does not interfere with DNA recovery. Adjust the volume of Buffer BL required based on the amount of starting material.

1. Add 220 μL absolute ethanol (96-100%, room temperature). Vortex to mix.

**Note：**Adjust the volume of ethanol required based on the amount of starting material.

1. Place the **DNA Mini Column** into the collection tube. Transfer the entire sample from step **6** into the column including any precipitate that may have formed. Centrifuge at 10,000 ×g for 1 minute. Discard the flow-through liquid.
2. Place the **DNA Mini Column** into the collection tube and add **500 μL Buffer KB**. Centrifuge at 10,000 ×g for 1 minute. Discard the flow-through liquid.
3. Place the **DNA Mini Column** into the collection tube. Add **600 μL DNA Wash Buffer**.Centrifuge as above and discard flow-through liquid.

**Note：**Ensure that absolute ethanol has been added to the DNA Wash Buffer before use. See label for directions. If refrigerated, DNA Wash Buffer must be brought to room temperature before use.

1. Using the same **2 mL Collection Tube**, repeat step **9**.
2. Using the same **2 mL Collection Tube**, and centrifuge with the lid open at maximum speed (≥12,000 ×g) for 2 minutes to remove the residual ethanol.

**Note：**This step is crucial for ensuring optimal elution.

1. Place the **DNA Mini Column** into a sterile 1.5 mL centrifuge tube, and add **100-150 μL** preheated (65℃) **Elution Buffer**. Allow to sit at room temperature for 1-3 minutes. Centrifuge at ≥13,000 ×g for 1 minute.
2. **Optional:** Add the eluted DNA back to the column for a second elution normally yields another 20-30% of the DNA while the first elution typically yields 60-70% of the DNA.

**Note：**Elution volume < 50 μL greatly reduces yield. Yields may be increased by incubating the column at 65℃ (rather than at room temperature) upon the addition of Elution Buffer.

1. The total DNA yield can be determined by a spectrophotometer using deionized water, Tris-HCl Buffer, or Elution Buffer as a blank. Dilute the DNA in TE buffer and calculate the concentration using the following equation:

**[DNA] = (Absorbance 260) × (0.05 μg/μL) × (Dilution Factor)**

The quality of DNA can be assessed by measuring absorbance at both 260 nm and at 280 nm. A 260/ 280 ratio of 1.7-1.9 corresponds to 85%-95% purity.

# B: Culture Cell gDNA Isolation Protocols

This method can quickly isolate approximately 25 μg of genomic DNA from 5 x 106 culture cells.

1. Prepare the cell suspension.
2. If the sample is frozen, thawed the sample, collect the cells by centrifugation, wash the cells in PBS buffer, and resuspend the cells in **200 μL Buffer TL**.
3. If the sample is suspended cells, collect 5 x 106 cells by centrifugation at 1,200 ×g, discard the supernatant, wash the cells in PBS buffer, and resuspend the cells in **200 μL Buffer TL**.
4. If the sample is a monolayer of growing cells, scrape the cells with a rubber sheet and wash the cells twice with PBS buffer. Resuspend the cells in **200 μL Buffer TL**.
5. Add **25 μL** of **Proteinase K**, vortex to mix, and stand at 50℃ water bath to facilitate complete tissue lysis.

**Optional:** If the cultured cells contain large amounts of RNA, the RNA needs to be removed during this process, excluding its effect on PCR. Add 4 μL of RNase A (20 mg/mL) and incubate at room temperature for 2 minutes.

1. Add **220 μL** **Buffer BL**, vortex and Incubate the tube at 50℃ for 10 minutes.

**Note:** After adding Buffer BL, fibrous precipitate may form without affecting DNA purification.

1. Add 220 μL absolute ethanol (96%-100%, at room temperature). Mix by vortexing.
2. Place the **DNA Mini Column** into a **2 mL Collection Tube**. Transfer the sample from step **4** including precipitate to the **DNA Mini Column**, and centrifuge at 10,000 ×g for 1 minute. Discard the flow-through liquid and put the column back to the collection tube.
3. Add **500 μL Buffer KB** into **DNA Mini Column** and centrifuge at 10,000 ×g for 1 minute, Discard flow-through liquid and reuse the collection tube.
4. Add **600 μL DNA Wash Buffer** into **DNA Mini Column** and centrifuge at 10,000 ×g for 1 minute. Discard flow-through liquid and put the column back to the collection tube.
5. Repeat step **7**.
6. Put the empty column, **with the lid open**, into the same collection tube and centrifuge at least 12,000 ×g for 2 minutes to dry the column.

**Note：**Residual ethanol will be removed more efficiently with the lid of the column open. It is critical to remove the ethanol before elution.

1. Place the **DNA Mini Column** into a sterile 1.5 mL microtube. Add **100-150 μL** preheated (65℃) **Elution Buffer**. Allow to sit at room temperature for 3 minutes. Centrifuge at 12,000 ×g for 1 minute.

**Optional:** Add the eluted DNA back to the column for a second elution normally yields another 20-30% of the DNA while the first elution typically yields 60-70% of the DNA.

# C: Mice Tail gDNA Isolation Protocols

Bring frozen samples and Proteinase K solution to room temperature, and pre-heat aliquots of Elution Buffer (0.5 mL / sample) at 65℃.

1. Cut two pieces of mouse tail 0.2-0.5 cm in length. Place into a sterile1.5 mL microcentrifuge tube, and add **180 μL Buffer TL**.If necessary cauterize the wound to stop bleeding. Having appropriately earmarked the animal, return it to a clean cage.

**Note:** The experimental mice should not exceed 6 weeks of age, otherwise the tissue samples are more difficult to lyse with lower DNA yield. Tail can be collected at 2-4 weeks of age and stored at -70℃ for genomic DNA extraction.

1. Add **25 μL** of **Proteinase K**, vortex to mix, and incubate in a 50℃ water bath with shaking for 1-4 hours to facilitate lysis of the tissue. If there is no shaking incubator, vortex the sample every 20-30 minutes.

**Note:** Incomplete lysis may block column flow and significantly reduce DNA yields. Tail lysis time depends on the length of the tail and the age of the animal. Incomplete lysis may block column flow and significantly reduce DNA yields.

**Optional:** mouse tail snips have low levels of RNA which will be co-purified with DNA using this kit. While it will not interfere with PCR, the RNA may be removed at this point. To do so, add 4 μL of RNase A (20 mg/mL) and incubate at room temperature for 2 minutes. Proceed with the following protocol.

1. Add **1 volume** of **Buffer BL**, 1 volume of absolute ethanol, mix well by vortexing.
2. Insert a **DNA Mini Column** into a **2 mL Collection Tube**, transfer the entire sample from Step **3** into the column including any wispy precipitate that may have formed.
3. Centrifuge at 10,000 ×g for 1minute. Discard flow-through liquid and put the column back to the collection tube.
4. Add **500 μL Buffer KB** into the **DNA Mini Column** and centrifuge at 10,000 ×g for1 minute, Discard flow-through liquid and reuse the collection tube.
5. Add **600 μL DNA Wash Buffer***.* Centrifuge at 10,000 ×g for 1 minute. Discard flow-through liquid and put the **DNA Mini Column** back to the collection tube.
6. Repeat step **7**.
7. Put the empty column, **with the lid open**, into the same collection tube and centrifuge at least 12,000 ×g for 2 minutes to dry the column.

**Note：**Residual ethanol will be removed more efficiently with the lid of the column open. It is critical to remove the ethanol before elution.

1. Insert the DNA Column into a new 1.5 mL centrifuge tube and add **100-200 μL** of pre-heated (65℃) **Elution Buffer** to the **DNA Mini Column** and allow to stand at room temperature for 3 minutes. Centrifuge at 12,000 ×g for 1 minute to elute the DNA.

**Optional:** Add the eluted DNA back to the column for a second elution normally yields another 20-30% of the DNA while the first elution typically yields 60-70% of the DNA. Elution volume < 50 μL greatly reduces yields. Yields may be increased by incubating the column at 65℃ (rather than at room temperature) upon the addition of Elution Buffer.

# D: FFPE gDNA Isolation Protocols

1. Place less than 30 mg of tissue (2~3 mm3) into a 2 mL centrifuge tube.
2. Add 1 mL of xylene (not supplied) to remove paraffin and vortex to mix well.
3. Centrifuge at 10,000 ×g for 10 minutes (at room temperature). Discard flow-through liquid.
4. Add 1 mL of absolute ethanol to remove the remaining xylene. Centrifuge at 10,000 ×g for 5 minutes at room temperature. Carefully pour off the ethanol to ensure that the pellet is intact.
5. Repeat with step **4**.
6. Dry the tissue pellet in air at 37℃ for 15 minutes.
7. Add **200 μL Buffer TL**, follow standard procedures for tissue DNA extraction and proceed from step **2 on page 4**.
8. For elution of DNA, it is recommended to use **50-100 μL** of Preheated **Elution Buffer** at 65℃. The amount of DNA harvested depends on the tissue size and age of the sample and can be increased by increasing the duration of TL buffer action.

**Note:** If paraformaldehyde is used to fix the tissue, the DNA and RNA in the tissue will degrade. Selecting different fixatives to preserve the specimen, the length of the DNA fragments contained in the specimen will change. These fragments are often less than 500 bp. This degradation phenomenon is that the DNA fragments in the tissues themselves have been degraded rather than caused during the extraction process. If the target gene fragment is less than 500 bp, good results can be obtained with the PCR method.

# Trouble Shooting Guide

|  |  |  |
| --- | --- | --- |
| **Problems** | **Possible Reasons** | **Suggested Improvements** |
| Clogged column | Incomplete lysis. | Add the correct volume of Buffer BL and incubate for specified time at 70℃. It may be necessary to extend incubation time by 10 minutes. |
| Sample is too large. | If using more than 30 mg of tissue, increase volumes of Proteinase K, Buffer BL. Pass aliquots of lysate through one column successively. |
| Sample is too viscous. | Divide sample into multiple tubes. |
| Low DNA yield | Clogged column. | See above. |
| Poor elution. | Repeat elution or increase elution volume (see notes on elution on page **7**) Incubation of column at 65℃ for 5 minutes with Elution Buffer may increase yields. |
| Improper washing. | Wash Buffer Concentrate must be diluted with absolute (100%) ethanol as specified on page 3 before use |
| Buffy coat used. | With buffy coat samples, use absolute ethanol, rather than isopropanol |
| 260 /280Low A /A ratio | Extended centrifugation during elution. | Resin from the column may be present in elute and affect the OD absorbance. Avoid centrifugation at speed higher than 15,000 rpm. The trace resin in the eluted DNA will not interfere with PCR or restriction digests |
| Poor cell lysis due to incomplete mixing with Buffer BL. | Repeat the procedure, this time making sure to vortex the sample with Buffer BL immediately and completely |
| Hemoglobin remains on column. | After application of sample to the column, wash once with 2 mL of Buffer BL. |
| No DNA eluted | Poor cell lysis due to improper mixing with Buffer BL. | Mix thoroughly with Buffer BL prior to loading the column. |
| Absolute ethanol not added to Buffer BL. | Before applying the sample to the column and aliquot of Buffer BL/ethanol solution must be added. |
| No ethanol added to DNA Wash Buffer concentrate. | Dilute DNA Wash Buffer with the indicated volume of absolute ethanol before use (page **3**). |
| Washing leaves colored residue in column | Incomplete lysis due to improper mixing with Buffer BL. | Buffer BL is viscous and the sample must be mixed thoroughly. |
| No ethanol added to DNA Wash Buffer concentrate | Dilute DNA Wash Buffer with the indicated volume of absolute ethanol before use. |
| Eluted material has red or brown color | Sample is too large | Reduce sample, and proceed with protocol. |
| Hemoglobin remains in column | After applying sample, wash column once with 3 mL of Buffer BL. |

# 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.beiwobiomedical.com](http://www.biomiga.com.cn/)