***Ver: 2402***

**Blood gDNA Isolation Kit (Column)**

**（BW-GD2311）**

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# Kit Contents

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| **Catalog#** | **BW-GD2311-00** | **BW-GD2311-01** | **BW-GD2311-02** |
| Preps | 4 | 50 | 250 |
| DNA Mini Columns | 4 | 50 | 250 |
| 2 mL Collection Tubes | 4 | 50 | 250 |
| Buffer BL | 1.2 mL | 15 mL | 65 mL |
| Buffer KB | 2.1 mL | 26 mL | 130 mL |
| Red Blood Cell Lysis Buffer | 10 mL | 125 mL | 2 x 300 mL |
| DNA Wash Buffer\* | 2 mL | 15 mL | 3 x 24 mL |
| Elution Buffer | 1.5 mL | 13 mL | 63 mL |
| Proteinase K | 110 μL | 1.3 mL | 5 x 1.3 mL |
| RNase A (20 mg/mL) | 25 μL | 270 μL | 1.4 mL |
| User Manual | 1 | 1 | 1 |

\*Add 8 mL (BW-GD2311-00) or 60 mL (BW-GD2311-01) or 96 mL (BW-GD2311-02) 96-100% ethanol to each DNA Wash Buffer bottle before use.

# Introduction

The Blood gDNA Isolation Kit provides a fast and easy method for isolating gDNA from blood. The system utilizes the reversible nucleic acid-binding properties of Biomiga’s membrane and the speed of spin column technology to yield high quality gDNA. Up to 250 μL of fresh, frozen or anticoagulated whole blood can be readily processed at one time. This kit can also be used for the preparation of genomic DNA from buffy coat, serum, plasma, saliva, buccal swab and other body fluids. Purified DNA is ready for applications such as PCR, Southern Blotting, and Restriction Digestion.

The binding capacity per column is 100 μg of gDNA. Use less than 250 μL of whole Blood or buffy coat is recommended.

# Storage and Stability

Proteinase K and RNase A is stable at room temperature (15-25℃) for one year. For long term, Proteinase K should be stored at -20℃. All other materials can be stored at room temperature (15-25℃). The guaranteed shelf life is 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 and pay special attention to the followings.

# Important Notes

❂ Add 8 mL (BW-GD2311-00) or 60 mL (BW-GD2311-01) or 96 mL (BW-GD2311-02) 96-100% ethanol to each DNA Wash Buffer bottle before use.

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

❂ Ensure the availability of centrifuge capable of 12,000 ×g.

❂ *Carry out all centrifugations at room temperature.*

# Materials not Supplied

❂ High speed microcentrifuge and Sterile 1.5 mL centrifuge tubes.

❂ 100% ethanol.

❂ Water bath

❂ 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 waste. Wear gloves and protective eyewear when handling.

# Whole Blood gDNA Mini Isolation Protocols

This method is suitable for the extraction of genomic DNA from fresh or frozen blood samples up to 250 μL. Genomic DNA can also be extracted from blood samples containing anticoagulants, buffy coats, serum, saliva, buccal swabs, and other bodily fluids.

1. Transfer the sample into a 1.5 mL centrifuge tube. If the sample volume is less than **250 μL,** add PBS or **Elution Buffer** (supplied) to 250 μL.

**Note:** If the sample volume is more than 250 μL, add **1 to 2.5 volumes** of **Red Blood Cell Lysis Buffer** (provided) and invert 5 times to mix. Centrifuge at 10,000 ×g for 1 minute, aspirate the supernatant, add 250 μL of PBS and invert several times to mix.

**Note:** When purifying blood genomic DNA from birds or amphibians, the sample volume needs to be reduced to 5-25 μL. Add PBS or Elution Buffer (provided) to 250 μL.

1. Add **25 μLProteinase K** and **250 μLBuffer BL**. Mix well by vortexing at maximum speed for 10 seconds. If RNA-Free genomic DNA is required, add **5 μLRNase A** (*20 mg/mL*) to each sample.
2. Incubate the sample at 50℃ for 20 minutes. Briefly vortex the tube once during incubation.
3. Add 250 μL absolute ethanol to the lysate. Vortex at maximum speed for 10 seconds and briefly centrifuge the tube to collect any drops from the lid.
4. Place the **DNA Mini Column** into a **2 mL Collection Tube**. Transfer the sample to the **DNA Mini Column**, and centrifuge at 12,000 ×g for 30 seconds. Discard flow-through liquid and put the column back to the collection tube.
5. Add **500 μLBuffer KB** into the **DNA Mini Column**, and centrifuge at 12,000 ×g for 30 seconds. Discard flow-through liquid and put the column back to the collection tube.
6. Add **600 μLDNA Wash Buffer** (*Add ethanol to DNA Wash Buffer before use*). Centrifuge at 12,000 ×g for 30 seconds. Discard flow-through liquid and put the **DNA Mini Column** back to the collection tube.

**Optional:** Repeat step **7**.

1. Reinsert the spin column, with the lid open, into the collection tube and centrifuge for 2 minutes at 12,000 ×g to dry the column.

**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 Mini Column** into a sterile 1.5 mL tube and add**50-150 μL** of pre-heated (70℃) **Elution Buffer** into the center of the column and let it stand for 5 minutes. Elute the DNA by centrifugation at 12,000 ×g for 1 minute.

**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.

1. The DNA concentration can be calculated as follows,

**Concentration (μg/mL)=OD260×50×dilution factor.**

The quality of DNA can be assessed by measuring absorbance at both 260 nm and at 280 nm. A260/280 ratio of 1.7-1.9 corresponds to 85%-95% purity. Expected yields will range from 4 μg to 12 μg of DNA per 250 μL of whole blood, depending on the source of the sample, its age, and the method of storage. Yields are generally 5-fold higher with buffy coat samples.

# Anticoagulant knowledge

1. **Sodium citrate (sodium citrate)**: small side effects, the anticoagulant effect is general, red blood cell sedimentation is easy to occur, and the preservation period is not long.
2. **EDTA**: Easy configuration, EDTA salt has little effect on red and white blood cell morphology. In the subsequent genomic DNA extraction test, the highest yield was obtained in the comparison of the three anticoagulant-preserved bloods, because the change of blood cell morphology in the EDTA anticoagulant was small, which facilitates the lysis. The blood preservation period is longer.
3. **Heparin**: Although heparin can maintain the natural form of red blood cells, it can often cause leukocyte aggregation. Heparin is an ideal anticoagulant for red blood cell permeability percolation tests. In addition, heparin residues have a certain influence on downstream molecular biology experiments.

# 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 65℃. It may be necessary to extend incubation time by 10 minutes. |
| Sample is too large | If using more than 10 mL Blood, 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 Yield | Clogged Column | See above. |
| Poor elution | Repeat elution or increase elution volume. Incubation of column at 70℃ 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. |
| Low Ratio of A260/280 | 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 ×g. 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 DNAEluted | 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 Wash Buffer Concentrate.  | Dilute 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 Wash Buffer Concentrate.  | Dilute Wash Buffer with the indicated volume of absolute ethanol before use.  |
| Eluted Material has Red or Brown Color | Sample Volume is too Large.  | Reduce sample volume, 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/)