**EZgeneTM Blood gDNA Midiprep Kit**

**（BW-GD2312）**

Contents

[Kit Contents 2](#_Toc130462344)

[Introduction 2](#_Toc130462345)

[Storage and Stability 2](#_Toc130462346)

[Before Starting 2](#_Toc130462347)

[Important 3](#_Toc130462348)

[Safety Information 3](#_Toc130462349)

[EZgeneTM Whole Blood DNA Midi Isolation Protocols 4](#_Toc130462350)

[Determination of Yield and Quality 6](#_Toc130462351)

[Trouble Shooting Guide 7](#_Toc130462352)

# Kit Contents

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| **Catalog #** | **BW-GD2312-00** | **BW-GD2312-01** | **BW-GD2312-02** |
| Preps | 2 | 10 | 25 |
| ezBind DNA Midi Columns | 2 | 10 | 25 |
| 15 mL Collection Tubes | 4 | 20 | 50 |
| Buffer BL | 11 mL | 52 mL | 130 mL |
| Buffer KB | 11 mL | 52 mL | 130 mL |
| 10 x Red Blood Cell Lysis Buffer | 14 mL | 70 mL | 175 mL |
| DNA Wash Buffer | 3.5 mL | 24 mL | 54 mL |
| Elution Buffer | 2mL | 10 mL | 25 mL |
| Proteinase K | 550 μL | 2.6 mL | 6.5 mL |
| RNase A (20 mg/mL) | 25 μL | 110 μL | 275 μL |
| Instruction Booklet | 1 | 1 | 1 |

# Introduction

The EZgeneTM Blood midi gDNA kit provides a fast and easy method for isolating gDNA from blood. The system utilizes the reversible nucleic acid- binding properties of Biomiga’s ezBind membrane and the speed of spin column technology to yield high quality gDNA with the OD260/280 ratio of 1.8-2.0 Up to 10 mL of fresh, frozen or anticoagulated whole blood treated with any common anticoagulant such as heparin, EDTA, or acid-citrate-dextrose can be readily processed at one time. This DNA 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 500 μg of gDNA. Use less than 10 mL of whole Blood or buffy coat is recommended.

# Storage and Stability

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

* + - Protease K store at -20°C.
    - All other materials at room temperature（4-25℃） .

# 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

* **Dilute DNA Wash Buffer with absolute ethanol as follows: Add 14 mL (GD2312-00) or 96 mL (GD2312-01) or 216 mL (GD2312-02) of absolute ethanol to each bottle**. The final concentration is 80%.
* Under cool ambient conditions, precipitates may form in Buffer BL. In case of such an event, heat the bottle at 37°C to dissolve before use.
* Preheat the Elution Buffer at 70°C **. Materials provided by user**
* Centrifuge capable of 5,000 x g
* Sterile 15 mL centrifuge tubes
* Water bath set to 55°C and 70°C
* Absolute ethanol

# 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, ware gloves and protective eyewear when handling.

# EZgeneTM Whole Blood DNA Midi Isolation Protocols

Calculate the amount of 10 x Red Blood Cell Lysis Buffer to be used. Mix one part of 10 x Red Blood Cell Lysis Buffer with nine part of ddH2O before use.

Final concentration of Red Blood Lysis Buffer:

NH4Cl 155 mM

KHCO3 10 mM

Na2EDTA 0.1 mM

Adjust to pH7.4 with 1 M HCl or NaOH

1. Transfer **2 mL whole blood sample** (up to 10 mL) into a tube and add **5 volumes** of **Red Blood Lysis Buffer**. For example, add 10 mL Red Blood Lysis Buffer to 2 mL whole blood. Mix by vortexing.

**Note:** Red Blood Cell Lysis Buffer is supplied as a 10 x concentrate and must be diluted with ddH2O according to bottle label before use.

1. Incubate for 15 to 20 min on ice, mixing by brief vortexing twice. The solution will become translucent.
2. Centrifuging at 450 g for 10 min at 4°C to precipitate the leukocytes. Discard the supernatant containing lysed red blood cells.
3. Wash the leukocytes with **2 volumes** of **Red Blood Lysis Buffer** of sample used in step 1. For example, add **10 mL Red Blood Lysis Buffer** if **5 mL** sample is used.
4. Centrifuging at 450 g for 10 min at 4°C again to precipitate the leukocytes. Discard the supernatant containing lysed red blood cells.
5. Add **0.25 mL** of **Protease K**. If RNA-Free genomic DNA is required, add **10 μL** of **RNase A** (provided) to each sample and mix briefly by vortexing. **Add 5 mL** of **Buffer BL**. Mix well by vortexing at maximum speed for 30s.

Note: Precipitates may form in Buffer BL. Dissolve at 37°C before use.

1. Incubate the sample at 50°C for 20 minutes. Briefly vortex the tube once during incubation.
2. Add **2.8 mL absolute ethanol** to the lysate. Vortex at maximum speed for 10s. Briefly centrifuge the tube to collect any drops from the lid.
3. Place the Midi column into 15 mL tube. Transfer up to **3mL** sample from step 8 to the DNA midi column, and centrifuge at 4,000 g for 5 min to bind DNA. Discard flow-through liquid and reuse the collection tube. apply the remaining of mixture into the column and centrifuge as above.
4. Place the column into the same collection tube. Add **5 mL** of **Buffer KB,** and centrifuge at 4,000 g for 5 min. Discard the collection tube and flow through liquid and put the column back to the collection tube.
5. Add **4 ml of DNA Wash Buffer** *(Add ethanol before use)****.*** Centrifuge at 4,000 g for 5 min. Discard flow-through liquid and put the column back to the collection tube.Repeat step 11 to wash the column again.
6. Put the empty column, **with the lid open**, into the same collection tube and centrifuge at 5,000 g for 15 min 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 column into a sterile new 15 mL microtube, add **300-800 μL Elution Buffer**. Incubate at 80oC for 5~10 min . Centrifuge at 10,000 g for 5 min to elute the DNA. Add another **500 μL** of **preheated Elution buffer** to elute. If maxium yield is required, please repeat wash for serval times with more elution buffer Combine the DNA eluant.

# Determination of Yield and Quality

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) x (0.05 μg/μL) x (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. Expected yields will range from 4 μg - 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.

# Trouble Shooting Guide

|  |  |  |
| --- | --- | --- |
| **Problem** | **Cause** | **Possible Solution** |
| **Clogged Column** | Incomplete Lysis | Add the correct volume of Buffer BL and incubate for specified time at 65°C . It may be necessary to extend  incubation time by 10 min. |
|  | Sample is too Large | If using more than 10 mL of Blood, increase volumes of Protease 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 6) Incubation of column at 70°C for 5 min  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 x 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 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 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 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 Biomiga’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 Biomiga. Biomiga’s sole obligation and purchaser’s exclusive remedy for breach of this warranty shall be, at the option of Biomiga, to replace the products, Biomiga 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.

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