***Ver.2306***

**EZgeneTM Mycoplasma gDNA Miniprep Kit**

**(BW-GD3211)**

# Contents

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# Kit Contents

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| **Catalog #** | **GD3211-00** | **GD3211-01** | **GD3211-02** |
| Preps | 4 | 50 | 250 |
| ezBind DNA Mini Columns | 4 | 50 | 250 |
| 2 mL Collection Tubes | 4 | 50 | 250 |
| Buffer LY | 1.2 mL | 15 mL | 65 mL |
| Buffer KB | 2.1 mL | 26 mL | 130 mL |
| DNA Wash Buffer | 2 mL | 15 mL | 3 x 24 mL |
| Elution Buffer | 2 mL | 15 mL | 60 mL |
| Proteinase K | 108 μL | 1.3 mL | 5 x 1.3 mL |
| RNase A | 25 μL | 270 μL | 1.4 mL |
| User Manual | 1 | 1 | 1 |

# Introduction

The EZgeneTM mycoplasma gDNA kit provides a fast and easy method for isolating gDNA from mycoplasma. 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 1.7-1.9. Purified DNA is ready for applications such as PCR, Southern Blotting, and Restriction Digestion.

# Storage and Stability

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

* Proteinase K is stable at room temperature ure(4-28°C ) for one year. For long term, store aliquots at ﹣20℃. Store all other contents at room temperature ure(4-28°C ).
* All other materials at room temperature(4-28°C ) .

# Materials provided by user

* Tabletop microcentrifuge
* Sterile 1.5 mL centrifuge tubes
* Water bath
* Absolute ethanol
* PBS Buffer

# 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 8 mL (GD3211-00) or 60 mL (GD3211-01) or 96 mL (GD3211-02)**

**of absolute ethanol to each bottle**. The final concentration is 80%.

* Under cool ambient conditions, precipitates may form in Buffer LY. In case of such an event, heat the bottle at 37°C to dissolve before use.

# EZgeneTM Mycoplasma DNA Isolation Protocols

All centrifugation steps must be carried out at room temperature

* 1. Transfer the sample to a 1.5 mL tube and bring the volume up to **250 μL** with

**PBS** or **Elution Buffer** (provided) if the sample volume is less than **250 μL**.

* 1. Add **25 μL** of **Proteinase K** and **250 μL** of **Buffer LY**. Mix well by vortexing at maximum speed for 10s. If RNA-Free genomic DNA is required, add **5 μL** of **RNase A(20 mg/mL)** (provided) to each sample.

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

* 1. Incubate the sample at 50°C for 10 minutes. Briefly vortex the tube once during incubation.
	2. Add **260 μL of absolute ethanol** to the lysate. Vortex at maximum speed for 10s. Briefly centrifuge the tube to collect any drops from the lid.
	3. Insert a DNA column into a collection tube.Transfer the sample to the DNA column, and centrifuge at 12,000 rpm for 30 s. Discard flow-through liquid.
	4. Place the column back into the collection tube. Add **500 μL** of **Buffer KB,** and centrifuge at 12,000 rpm for 30s. Discard flow through liquid and put the column back to the collection tube.
	5. Add **600 μL** of **DNA Wash Buffer** *(Add ethanol before use)****.*** Centrifuge at 12,000 rpm for 30s. Discard flow-through liquid.

**Note:** DNA Wash Buffer is provided as a concentrate and must be diluted with absolute ethanol as indicated on the bottle, and on page 3.Repeat step 7 with

another **600 μL DNA Wash Buffer** and centrifuge as above. Discard the flow- through.

* 1. Put the empty column, **with the lid open**, into the same 2 mL collection tube and centrifuge at 12,000 rpm for 1 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 1.5 mL microtube, add **100 μL** (preheated at 65°C) **Elution Buffer**. Incubate at room temperature for 2 min.
	2. Centrifuge at 13,000 rpm for 1 min to elute the DNA. The first elution normally yields 60-70% of DNA bound.
	3. **Optional:** Elute the column with another **100 μL** (preheated at 65°C) **Elution Buffer.** The second elution will yield another 20% of the DNA bound.

# 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.

# 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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