RUO

WizMag™ Bacterial DNA

User Manual

Ver 2.0

REF W7110 | W7111 | W7112 | W7113

For Research Use Only

wizbiosolutions
THE INNOVATION OF GENOMICS

INTENDED USE

The WizMag™ Bacterial DNA kit is designed to be used on the CLEO™ AP16 Nucleic Acid Extractor System for simple and easy purification of total DNA from the bacterial cultures of gram-positive bacteria or gram-negative bacteria. This kit utilizes the silica beads for the disruption of bacterial cell walls and the bead beating can be performed on any vortex that has a 2 mL tube-holder adapter. Purified DNA is free of enzyme inhibitors and other contaminants, and highly suited for downstream applications such as PCR-based or enzyme-based reactions.

KIT CONTENTS

Contents	W7110	W7111	W7112	W7113	Storage
No. of preparation	64	192	32	96	Room Temperature (15-25°C)
Pre-packed 96 well Plate	4 ea	12 ea	-	-	
Pre-packed 6-well Strip	-	-	32 ea	96 ea	
Plunger	8 ea	24 ea	8 ea	24 ea	
Powerbead™ B1 tube	64 ea	192 ea	32 ea	96 ea	
Buffer MM1	30 mL	90 mL	16 mL	45 mL	
Buffer MM2	5 mL	12 mL	3 mL	7 mL	
Buffer MM3	10.8 mL	31.5 mL	6.3 mL	16.2 mL	
Enhancing solution P	1.2 mL	3.5 mL	0.7 mL	1.8 mL	
RNase A solution*	120 µL	360 µL	64 µL	180 µL	
Blank solution A	500 µL	500 µL	500 µL	500 µL	

This kit is delivered under ambient conditions. When being used immediately on arrival, all the components can be stored at room temperature (15 - 25 °C). But if the kit is going to be stocked for a long time, Proteinase K should be stored at 2 - 8°C for optimal conservation. Long exposure to heat sources can deteriorate the performance of the kit significantly.
* Before first use, add all of RNase A solution into Buffer MM1, and store at 4°C.

QUALITY CONTROL ANALYSIS

In accordance with Wizbiosolutions Inc. ISO 13485-certified Quality Management System, each lot WizMag™ Bacterial DNA kit is tested against predetermined specifications to ensure consistent product quality.

PRECAUTIONS

- This product is for research use only.
- Intended for single use only. Do not reuse.
- Check the expiration date on the box. Do not use it after the expiration date.
- Wear protective clothing, and use disposable gloves, goggles, and a mask.
- Do not eat, drink or smoke in areas where samples or test reagents are being used. Once you finish the test wash your hands.
- Specimens must be treated as potentially infectious as well as all reagents and materials that have been exposed to the samples and handled in the same manner as an infectious agent.

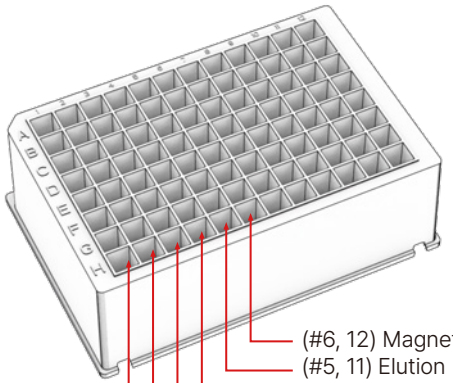
- Regular decontamination of commonly used equipment is recommended, especially micropipettes and work surfaces.
- This product contains irritants that are harmful when in contact with skin or eyes, or inhaled or swallowed. Care should be taken when handling this product.
- Some of the reagents in the 96-well Plate contain chaotropic which can form highly reactive compounds when combined with bleach. Do NOT add bleach or acidic solutions directly to the sample-preparation waste.
- Any significant incidents related to the product should be notified to the competent authorities and manufacturers.
- Do not use it if the package is damaged.

RECONSTITUTION OF PROTEINASE K

Before the first experiment, dissolve completely Proteinase K with Buffer PKR, as indicated on the product label. Do not vortex when dissolving. Store the reconstituted Proteinase K solution at 2 - 8°C.

COMPOSITION OF THE PRE-PACKED 96-WELL PLATE (W7110 | W7111)

A total of 16 samples can be simultaneously processed per plate.



Columns 7 - 12 in the right half of a 96-well plate have the same composition as columns 1 - 6 in the left half.

(#6, 12) Magnetic bead
(#5, 11) Elution
(#2/3/4, 8/9/10) Washing
(#1,7) Sample

COMPOSITION OF THE PRE-PACKED 6-WELL STRIP (W7112 | W7113)

(#1) Sample
(#2,3,4) Washing
(#5) Elution
(#6) Magnetic bead

PROTOCOL

▶ Check Before First Use

1. Preparation of buffer MM1 containing RNase A

Before first use, add all of RNase A solution into buffer MM1 and mix well by gentle swirling. Store RNase-containing buffer MM1 at 2 - 8 °C for conservation of activity.

2. Preparation of buffer MM3 with enhancing solution P

Before first use, add all of the enhancing solution P into buffer MM3 and mix well by gentle swirling. Store the Buffer MM3 at room temperature (20 - 25 °C).

A. Setup of program (For CLEO™ AP16 & AP48 devices, preset program can be used.)

Edit and run the experiment program as follows:

No.	1	2	3	4	5	6	7
Well #	6	1	2	3	4	5	6
Step	Beads	Bind	Wash	Wash	Wash	Elute	Discard
Wait time	-	-	-	-	-	03:00	-
Mix time	00:20	10:00	02:00	01:00	01:00	05:00	00:15
Collect time	00:25	00:30	00:25	00:25	00:25	00:30	-
Volume(µL)	200	900	750	750	750	100	200
Mixing speed	Medium	Fast	Fast	Fast	Fast	Bottom	Medium
Collect speed	Strong	Strong	Strong	Strong	Strong	Strong	Normal
Lysis temp.		Off					
Elute temp.						60°C	

B. Sample Preparation

- Prepare a 1.5 mL microcentrifuge tube and 65°C water bath or dry bath.
- Before first use, add all of RNase A solution into Buffer MM1, and store the buffer MM1 at 4°C.

1. Gram Positive Bacteria
   a. Harvest up to 2 x 10^9 bacterial cells in a 1.5 mL microcentrifuge tube by centrifugation and discard the supernatant as much as possible.
   b. Resuspend the pellet thoroughly in 400 µL of Buffer MM1.
      - For efficient resuspending, it is helpful to loosen and disrupt the pellets by flickering or vortexing before the addition of Buffer MM1.
      - Check that RNase A solution has been added to buffer MM1 before first use.
   c. Transfer all of the resuspension into the Powerbead™ B1 tube and mount the tube on the tube-holder adapter of the vortex machine or bead-beating machine.
   d. Vortex the tube on the highest setting (>2,000 rpm) for 10 minutes.
      - Required processing time will vary depending on the disrupting device and application and therefore should be evaluated on a case-by-case basis.

- For example, processing times may be as little as 1 minute when using high-speed cell disrupting machines (e.g., GeneReady™, FastPrep-24™ Precellys™ 24, PowerLyzer™ 24) or as long as 15 minutes when using lower speeds. See the manufacturer's literature for operating information.

e. Apply 50 µL of Buffer MM2 into the tube, vortex to mix thoroughly and incubate the tube at 65°C for 10 minutes.

f. Cool the tube to room temperature, add 150 µL of Buffer MM3 and vortex to mix thoroughly.
   - Check that the Enhancing solution P has been added to buffer MM3 before first use.

g. Centrifuge at >13,000 xg for 3 minutes.

h. Use300 µL of the supernatant as a sample.

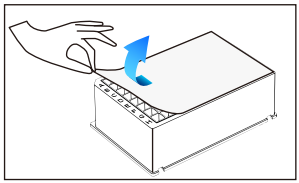
2. Gram Negative Bacteria

a. Harvest up to 2 x 10^9 bacterial cells in a 1.5 mL microcentrifuge tube by centrifugation and discard the supernatant as much as possible.

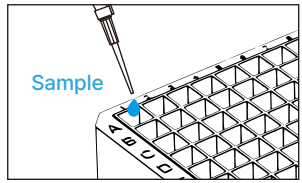
b. Resuspend the pellet thoroughly in 400 µL of Buffer MM1.
   - For efficient resuspending, it is helpful to loosen and disrupt the pellets by flickering or vortexing before the addition of Buffer MM1.
   - Check that RNase A solution has been added to buffer MM1 before first use.

c. Go to step e of A. Gram Positive Bacteria procedure.

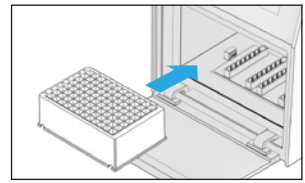
C-1. DNA extraction procedure (W7110, W7111)



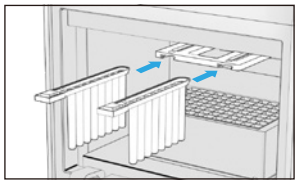
1. Carefully peel off the film of the 96-well Plate not to cross-contaminate.



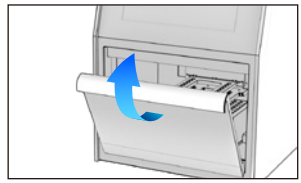
2. Apply 300 µL of the supernatant into the each first well (#1,7)



3. Mount the 96-well Plate on the CLEO™ AP16 carefully.

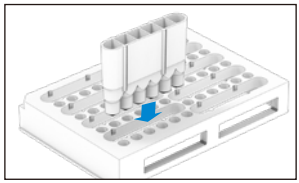


4. Insert a Plunger all the way into the socket above the 96-well Plate.

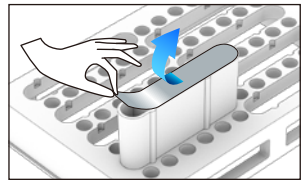


5. Close the front door of the instrument.

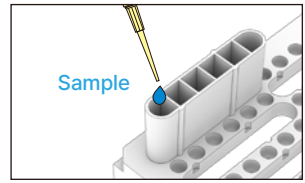
C-2. DNA extraction procedure (W7112, W7113)



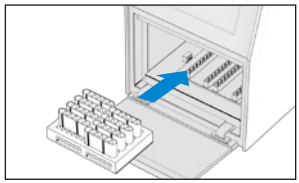
1. Mount the 6-well Strip onto the Strip Adapter Plate.



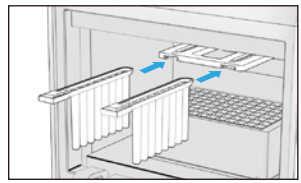
2. Carefully peel off the film of the 6-well Strip not to cross-contaminate.



3. Apply 300 µL of the supernatant into the each first well (#1)

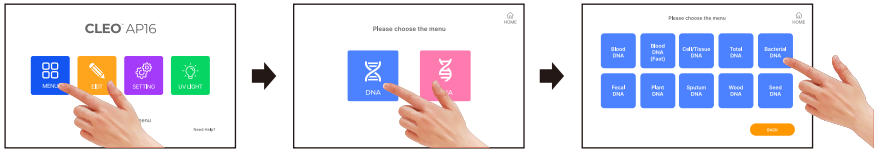


4. Mount the 6-well Strip Adapter Plate on the CLEO™ AP16 carefully.



5. Insert a Plunger all the way into the socket above the 96-well Plate.

6. Close the front door of the instrument.
7. Select **MENU** ▶ **DNA** ▶ **Bacterial DNA** on the screen.



8. Press **'RUN'** button on the screen.
9. After the alarm finishes, open the door and carefully remove the Plunger.
10. Detach the 96-well plate (or the Strip Adapter Plate) from the machine carefully.
11. Transfer the 60 - 80 µL eluate of each fifth well (#5,11) into a new 1.5 mL centrifuge tube.

    NOTE : The volume of eluate can be decreased slightly during the process.
12. Dispose of 96-well Plates (or 6-well Strip) and Plunger used in the test according to local or national waste disposal methods.

TROUBLE SHOOTING GUIDE

Problem	Possible causes	Recommendations
Low yield	Starting sample is not fresh or improperly stored	DNA yield may be reduced if the sample is improperly stored or outdated. Use a freshly harvested sample.
	Low beating power	Low beating power can't properly disrupt the cell wall of Gram(+) bacteria. Bead-beating should be carried out at maximum power on a conventional vortex machine. Please refer to the manufacturer's instruction manual for other disrupting machines.
Low purity	Co-transfer of debris	When transferring the sample mixture into the sample well, be careful not to co-transfer the debris of the pellet. This will lower the purity of DNA.
	Too much sample used	Do not overload the sample. Keep the maximum cell number in the sample as a procedure.
Degraded DNA	Too much power for bead-beating	Too high speed or longer time for bead-beating can cause the degradation of DNA. It is recommended to follow the basic procedure for the first time and then optimize the speed and the time for the sample at the next preparation.
	Starting sample is not fresh or improperly stored	DNA can be degraded if the sample is improperly stored or outdated. Use a freshly harvested sample if possible.
Inconsistent recovery of DNA	Contamination between reagent wells	The reagent in the well may evaporate and form a deposit on the film during storage, which may cause contamination between wells when the film is removed. Prepacked plates or tubes always should be stored in proper condition. Before removing the film from the plate or the tube, it is recommended to shake off the deposit on the film while holding the plate or the tube tightly.

SYMBOL GLOSSARY

REF	Catalogue number		Manufacturer		Use-by date
LOT	Batch code		Do not re-use		Temperature limitation
RUO	Research use only		Instructions for use		Keep away from sunlight
	Contents sufficient for <n> tests		Caution		Keep dry
	Do not use if package is damaged				

ORDERING INFORMATION

Product	Cat No.	Package	Note
WizMag™ Bacterial DNA	W7110	64 Prep	16 prep/run
	W7111	192 Prep	
	W7112	32 Prep	Single prep
	W7113	96 Prep	
CLEO™ AP16 Nucleic acid Extractor	CL2016	1 system	1-16 sample
CLEO™ AP48 Nucleic acid Extractor	CL2048	1 system	1-48 sample

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