RUO

WizMag™ Plasmid DNA

User Manual

Ver 1.0

REF W7140 | W7141 | W7142 | W7143

For Research Use Only

wizbiosolutions
THE INNOVATION OF GENOMICS

INTENDED USE

The WizMag™ Plasmid DNA kit is designed to easily prepare plasmid DNA from bacterial cultures on the CLEO™ AP16 Nucleic Acid Extractor System . This kit utilizes the alkaline lysis method to isolate plasmid DNA, which is purified by advanced magnetic silica technology. This kit can process up to 5 mL of E.coli cultures and is ready for endA$^+$ strains. Purified plasmid DNA can be directly used in various downstream applications, such as PCR, cloning, sequencing, and other enzymatic reactions, without any further manipulations

KIT CONTENTS

Contents	W7140	W7141	W7142	W7143	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	
Buffer MP1*	20 mL	55 mL	10 mL	30 mL	
Buffer MP2**	20mL	55 mL	10 mL	30 mL	
Buffer MP3**	30 mL	75 mL	13 mL	38 mL	
RNase A solution*	100 µL	275 µL	50 µL	150 µL	
Blank solution B	500 µL	500 µL	500 µL	500 µL	

This kit is delivered under ambient conditions. When being used immediately on arrival, all components can be stored at room temperature. But if the kit is going to be stocked for a long time, RNase A solution 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 MP1 and mix gently. Buffer MP1 should be stored at 2~8°C after the addition of RNase A solution.

** During shipment or storage under cold ambient conditions, a precipitate can be formed in Buffer MP2 and/or MP3. Heat the bottle at 20°C - 40°C to dissolve completely in such a case.

QUALITY CONTROL ANALYSIS

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

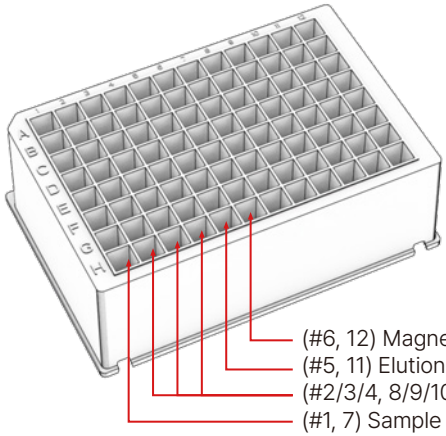
PRECAUTIONS

- 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.

COMPOSITION OF THE PRE-PACKED 96-WELL PLATE (W7140 | W7141)

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.

COMPOSITION OF THE PRE-PACKED 6-WELL STRIP (W7142 | W7143)

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

PROTOCOL

A. Setup of program (For CLEO™ AP16 & AP48 devices, preset program can be used.)
Edit and run the experiment program as follows:

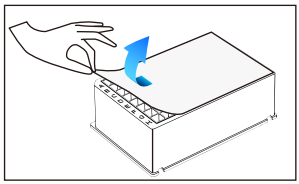
No.	1	2	3	4	5	6	7
Well #	6	1	2	3	4	5	6
Step	Beads	Bind	Wash	Wash	Wash	Elute	Discard
Wait time	-	-	-	-	-	03:00	-
Mix time	00:20	05:00	01:00	01:00	01:00	05:00	00:20
Collect time	00:25	00:30	00:25	00:25	00:25	00:45	-
Volume(µL)	200	750	750	750	750	70	200
Mixing speed	Medium	Fast	Fast	Fast	Fast	Bottom	Medium
Collect speed	Strong	Strong	Strong	Strong	Strong	Strong	Normal
Temperature		Off				60°C	

B. Sample Preparation

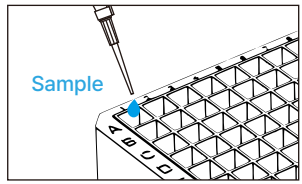
- Do not use a precipitated buffer. If a precipitate forms in a buffer, dissolve completely at 20 - 40°C before use.
- Before first use, add all of RNase A solution into the bottle of Buffer MP1 and mix well by gentle swirling. Store the Buffer MP1 at 2 - 8°C after the addition of RNase A solution.

1. Harvest up to 5 mL of bacterial culture by centrifugation for 3 minutes at 10,000 xg. Discard the supernatant as much as possible without disturbing the pellet.
2. Resuspend the pelleted cells thoroughly in 250 µL of Buffer MP1. Transfer the suspension to a new 1.5 mL microcentrifuge tube.
   - It is essential to thoroughly resuspend the cell pellet.
3. Add 250 µL of Buffer MP2 and mix gently by inverting until evenly blue. Let it stand until the mixture becomes translucent.
   - Do NOT vortex at this step and do NOT incubate the mixture for more than 5 minutes.
4. Add 350 µL of Buffer MP3 and mix immediately but gently by inverting until the mixture returns to evenly clear again.
5. Centrifuge for 10 minutes at full speed (>13,000 xg) and use the supernatant as a sample.

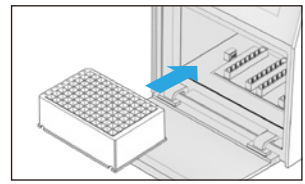
C-1. DNA extraction procedure (W7140, W7141)



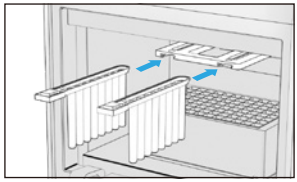
1. Carefully peel off the film of the 96-well Plate not to cross-contaminate.



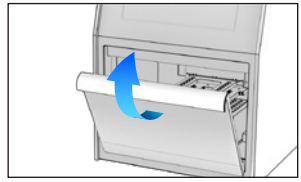
2. Apply 750 µL of the cleared supernatant into the each first well (#1, 7)



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

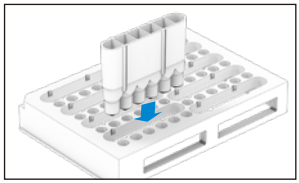


4. Insert a Plunger all the way into the socket above the 96-well Plate.

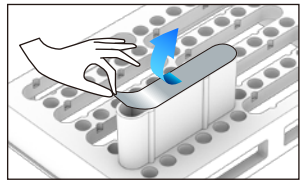


5. Close the front door of the instrument.

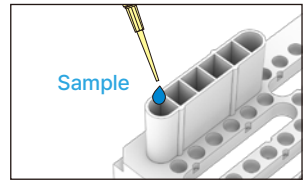
C-2. DNA extraction procedure (W7142, W7143)



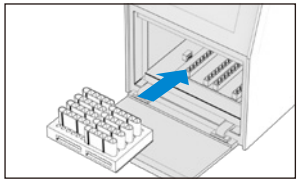
1. Mount the 6-well Strip onto the Strip Adapter Plate.



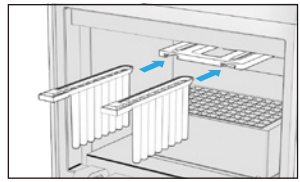
2. Carefully peel off the film of the 6-well Strip not to cross-contaminate.



3. Apply 750 µL of the cleared 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** ▶ **Plasmid 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 50 - 60 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.

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
Σ	Contents sufficient for <n> tests		Caution		Keep dry
	Do not use if package is damaged				

ORDERING INFORMATION

Product	Cat No.	Package	Note
WizMag™ Plasmid DNA	W7140	64 Prep	16 prep/run
	W7141	192 Prep	
	W7142	32 Prep	Single prep
	W7143	96 Prep	
CLEO™ AP16 Nucleic acid Extractor	CL2016	1 system	1-16 sample
CLEO™ AP48 Nucleic acid Extractor	CL2048	1 system	1-48 sample

TROUBLE SHOOTING GUIDE

Problem	Possible causes	Recommendations
Low recovery of plasmid DNA	Too many bacterial cells in the sample	Overloaded samples cannot be lysed efficiently, followed by poor yield of plasmid DNA. Bacterial cultures should be grown for 12-21 hours in proper media with antibiotics. Reduce the starting sample especially when rich broth is used, such as TB or 2xYT.
	Low copy-number plasmid targeted	Increase the starting sample with an additional buffer for low-copy number plasmid.
	Poorly resuspended bacterial pellets	Bacterial pellets should be re-suspended thoroughly in Buffer MP1. The clump of cells cannot be lysed efficiently.
	Buffer MP2 precipitated	Make sure the Buffer MP2 does not have any precipitates before use. Precipitated Buffer MP2 will lead to poor lysis of bacterial cells.
	RNA not digested	Excessive RNA can interfere with the binding of the plasmid to a column membrane. The Buffer MP1 containing RNase A should be stored at 2-8°C for the conservation of activity.
Low purity	Precipitates contaminated	Any cell debris or precipitates should not be co-transferred when applying the supernatant into the sample well.
Genomic DNA contaminated	Excessive handling after adding the Buffer MP3	Excessive or vigorous impact on the lysate may lead precipitated genomic DNA to be extruded into the solution. The Buffer MP3 added lysate should be handled carefully before centrifugation.
Plasmid DNA smeared	Excessive handling after adding the Buffer MP2	Excessive lysis time and/or vigorous vortexing of the lysate after the addition of Buffer MP2 will lead to irreversible denaturation of plasmid DNA. The lysis time should not be over 5 minutes and the lysate should be handled carefully.
RNA contaminated	RNase A omitted or inactive	All of the RNase A solution should be added to Buffer MP1 before first use. Buffer MP1 containing RNase A should be stored at 2~8°C. Add RNase A (final conc. 100 ug/mL) when the activity is decreased.
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.

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