IVD

WizMag™ Sputum DNA

User Manual

Ver 2.0

REF W7130 | W7131 | W7132 | W7133

For *in vitro* diagnostic use

wizbiosolutions
THE INNOVATION OF GENOMICS

INTENDED USE

The WizMag™ Sputum DNA kit is designed to be used on the CLEO™ AP16 and AP48 Nucleic Acid Extractor System for simple and easy purification of total DNA from the Sputum, bronchial or tracheal lavage. 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	W7130	W7131	W7132	W7133	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 PKR	1.5 ml	6 ml	1 ml	3 ml	
Proteinase K *	14 mg x 2	42 mg x 2	14 mg	42 mg	
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). However, if the kit will be stored for a long time, Proteinase K should be kept at 2 - 8°C for optimal preservation. Prolonged exposure to heat sources can significantly deteriorate the performance of the kit.

* After dissolving, store proteinase K solution at 2 - 8°C for optimal conservations.

QUALITY CONTROL ANALYSIS

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

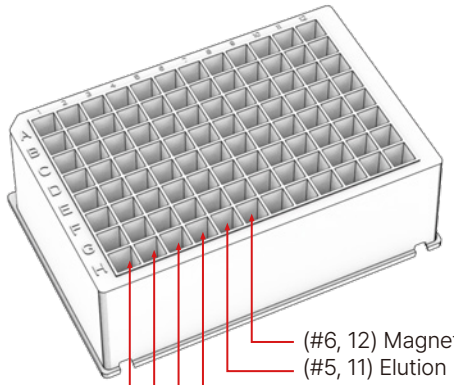
PRECAUTIONS

- This product is reserved exclusively for ***in vitro*** diagnostic purposes.
- 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 (W7130 | W7131)

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 (W7132 | W7133)

(#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:

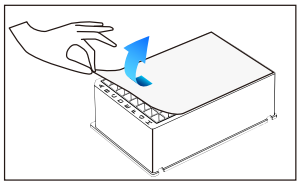
No.	1	2	3	4	5	6	7
Well #	6	1	2	3	4	5	6
Step	Beads	Lysis	Wash	Wash	Wash	Elute	Discard
Volume(µL)	200	600	750	750	750	100	200
Wait time	-	-	-	-	-	03:00	-
Mix time 1	00:15	10:00	00:10	00:10	01:00	00:15	00:15
Mix speed 1	Medium	Fast	Bottom	Bottom	Fast	Bottom	Bottom
Mix time 2	-	-	00:20	00:20	-	00:45	-
Mix speed 2	-	-	Fast	Fast	-	Fast	-
Mix loop	1	1	4	3	1	3	1
Collect time	00:15	00:20	00:15	00:15	00:15	01:00	-
Collect speed	Strong	Strong	Strong	Strong	Strong	Strong	Normal
Temperature		65°C				60°C	

B. Sample Preparation (Sputum or viscous BAL fluid)
The method for liquefying sputum samples varies among laboratories, and the current method is acceptable. The procedure detailed here is one of several standard techniques.

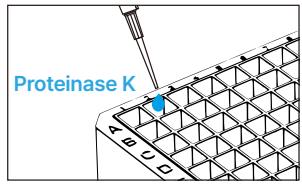
☞ Prepare a fresh 1N NaOH solution (4% NaOH solution).
☞ Prepare 1x PBS (pH 7.4).
☞ Prepare sterile water

1. **Collect a sputum sample into a 50 mL conical tube.**
   - Recommended sputum sample size: 1 mL or more
2. **Add an equal volume of a fresh 1N NaOH solution to the sputum sample, vortex to mix thoroughly, and let it stand until completely liquefied.**
   - For example, for 1 mL of sputum sample, mix with 1 mL of 1N NaOH solution.
   - It may take 10 to 30 minutes for complete liquefaction, depending on the sample.
3. **Add sterile water up to the 25 mL mark on the 50 mL tube, centrifuge at 3,000 xg for 10 minutes and carefully discard the supernatant.**
4. **Resuspend the pellet thoroughly with 1 mL of 1x PBS and transfer the mixture into a 1.5 mL microcentrifuge tube.**
5. **Centrifuge at 13,000 xg for 5 minutes, carefully discard the supernatant and resuspend the pellet with 1 mL of 1x PBS.**
6. **Centrifuge at 13,000 xg for 5 minutes, carefully discard the supernatant and resuspend the pellet with 200 uL of 1x PBS.**
7. **Use the 200 uL of the resuspended solution as a sample.**

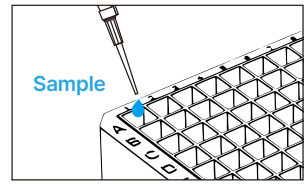
C-1. DNA extraction procedure (W7130, W7131)



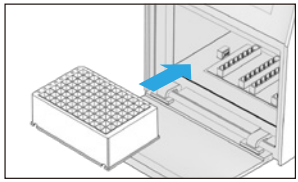
1. Carefully peel off the film of the 96-well Plate not to cross-contaminate.



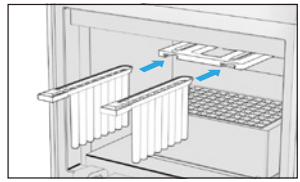
2. Add 20 µL of Proteinase K solution into the each first well (#1, 7)



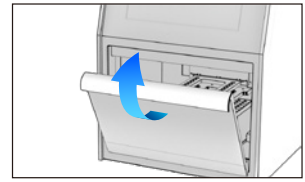
3. Apply 200 µL of the sample into the each first well (#1,7)



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

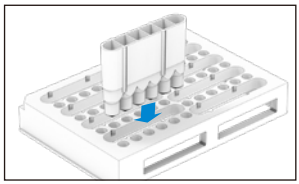


5. Insert a Plunger all the way into the socket above the 96-well Plate.

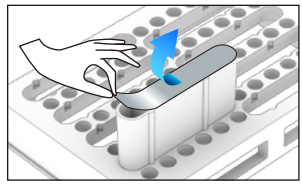


6. Close the front door of the instrument.

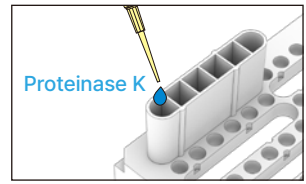
C-2. DNA extraction procedure (W7132, W7133)



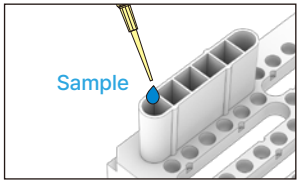
1. Mount the 6-well Strip onto the Strip Adapter Plate.



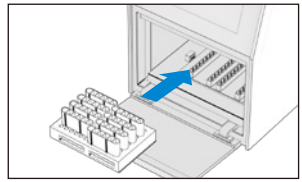
2. Carefully peel off the film of the 6-well Strip not to cross-contaminate.



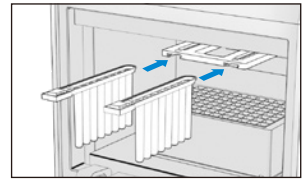
3. Add 20 µL of Proteinase K solution into the each first well (#1)



4. Add 200 µL of the sample into the each first well (#1).



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



6. Insert a Plunger all the way into the socket above the Plate.

7. Close the front door of the instrument.
8. Select **MENU** ▶ **DNA** ▶ **Sputum DNA** on the screen.

9. Press **'RUN'** button on the screen.
10. After the alarm finishes, open the door and carefully remove the Plunger.
11. Detach the 96-well plate (or the Strip Adapter Plate) from the machine carefully.
12. 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.
13. 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 or no recovery	Low cells in the starting sample	Some samples may have low population of cells. When the cell mass is very low, an addition of carrier RNA (5-10 µg/sample) into the sample can be helpful for the recovery of DNA.
	Too many cells in the starting sample	The sample amount over the maximum capacity will lead to poor lysis of cells, resulting in significantly low recovery. Reduce the amount of starting sample.
Low purity	Too much sample amount used	Do not overload the sample. Keep the volume and the cell number of sample as procedure.
High A_{260}/A_{280} ratio	RNA contamination	RNA can inhibit some downstream enzymatic reactions, but not PCR itself. If RNA should be removed from the preparation, add RNase A solution into well#2 at DNA recovery procedure. Alternatively, RNase A solution can be applied to the sample directly by 5 µL per sample.
Degraded DNA	Starting sample is too old or improperly stored	Too old or improperly stored samples may have degraded DNA. Use a fresh sample.
Inconsisten 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 plate or tube always should be stored at proper condition. Before removing the film of the plate or the tube, it is recommend-ed to shake off the deposit on the film with 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
IVD	in-vitro diagnostic use		Instructions for use		Keep away from sunlight
	Contents sufficient for <n> tests		Caution		Keep dry
	Do not use if package is damaged	UDI	Unique Device Identification		

ORDERING INFORMATION

Product	Cat No.	Package	Note
WizMag™ Sputum DNA	W7130	64 Prep	16 prep/run
	W7131	192 Prep	
	W7132	32 Prep	Single prep
	W7133	96 Prep	
CLEO™ AP16 Nucleic acid Extractor	CL2016	1 system	1-16 sample
CLEO™ AP48 Nucleic acid Extractor	CL2048	1 system	1-48 sample

MFDS License No. : IVD-24-6289

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