RUO

WizMag™ Fecal DNA

User Manual

Ver 2.0

REF W7120 | W7121 | W7122 | W7123

For Research Use Only

wizbiosolutions
THE INNOVATION OF GENOMICS

INTENDED USE

The WizMag™ Fecal DNA kit is designed to be used on the CLEO™ AP16 Nucleic Acid Extractor System for simple and easy purification of total DNA from human stool or animal fecal specimens. This kit utilizes the silica and zirconia beads for the disruption of bacterial or fungal cell walls in the sample and the bead beating can be performed on any disrupting device which has a 2 mL tube-holding adapter including a conventional vortex machine. 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	W7120	W7121	W7122	W7123	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™ F1 tube	64 ea	192 ea	32 ea	96 ea	
Buffer PFL	55 mL	150 mL	25 mL	70 mL	
Buffer PFP	10.8 mL	32.4 mL	5.4 mL	16.2 mL	
Enhancing Solution P	1.2 mL	3.6 mL	0.6 mL	1.8 mL	
RNase A solution*	150 µL	420 µL	80 µL	210 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™ Fecal DNA kit is tested against predetermined specifications to ensure consistent product quality.

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.

PRECAUTIONS

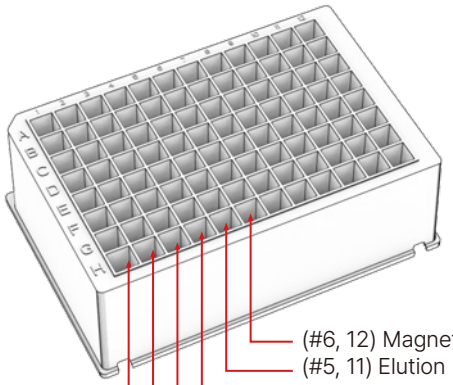
- This product is for research use only.
- Intended for single use only. Do not reuse.
- Check the expiration date on the box. Do not use it after the expiration date.
- Wear protective clothing, and use disposable gloves, goggles, and a mask.
- Do not eat, drink or smoke in areas where samples or test 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 (W7120 | W7121)

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 (W7122 | W7123)

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

PROTOCOL

▶ Check Before First Use

Preparation of Buffer PFP with enhancing solution P
Before first use, add all of the enhancing solution P into Buffer PFP and mix well by gentle swirling. Store the Buffer PFP 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	00:20
Collect time	00:25	00:30	00:30	00:30	00:30	00:35	-
Volume(µL)	200	700	750	750	750	100	200
Mixing speed	Medium	Fast	Fast	Fast	Fast	Bottom	Med
Collect speed	Strong	Strong	Strong	Strong	Strong	Strong	Normal
Temperature		Off				60°C	

B. Sample Preparation

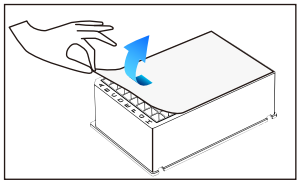
- Prepare a 1.5 mL microcentrifuge tube.

1. **Apply up to 250 mg of feces specimen and 700 µL of Buffer PFL into Powerbead™ F1-bead tube and briefly vortex.**
   - If the sample feces have low moisture content or are dried slightly, apply a lesser amount of sample.
2. **Secure the tube on a 2 mL tube holder of a vortex machine or a disrupting machine.**
3. **Vortex the tube on the highest setting (>2,000 rpm) for 10 minutes.**
   - Required processing time will vary depending on the device and application and therefore should be evaluated on a case-by-case basis.
   - For example, processing times may be as little as 2 minutes 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 (e.g., Vortex Genie™, or standard benchtop vortexes). See the manufacturer's literature for operating information.
4. **Centrifuge at >13,000g for 1 minute and transfer up to 450 µL of the supernatant to a new 1.5 mL centrifuge tube.**
   - The supernatant may still contain some fecal particles.
   - If the applied feces sample has low moisture content or is dried, less volume of supernatant may be available.
5. **Add 2 µL of RNase A solution (provided) to the sample tube, vortex to mix, and incubate for 2 minutes at room temperature.**

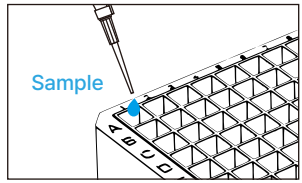
- Alternatively, RNase A solution can be added at step 1 with Buffer PFL without incubating times.

6. **Apply 150 µL of Buffer PFP and vortex for 5 seconds.**
   - If the volume of the transferred supernatant was less than 450 µL, apply 1/3 volume of PFP to a transferred volume.
   - Check that the enhancing solution P has been added to Buffer PFP before first use.
7. **Centrifuge at >13,000g for 3 minute.**
8. **Use 300 µL of the supernatant as a sample.**
   - Be careful not to co-transfer the debris.

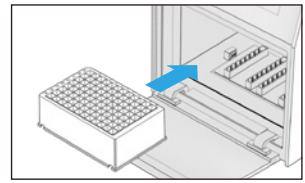
C-1. DNA extraction procedure (W7120, W7121)



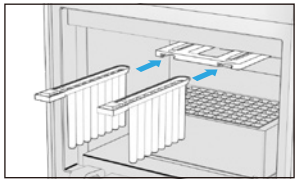
1. Carefully peel off the film of the 96-well Plate not to cross-contaminate.



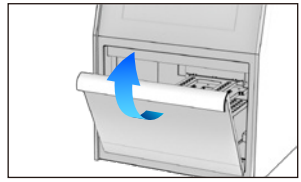
2. Add 300 µL of the sample into the each first well (#1,7)



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

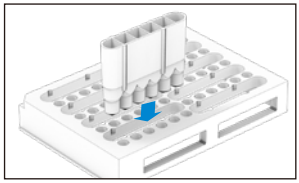


4. Insert a Plunger all the way into the socket above the 96-well Plate.

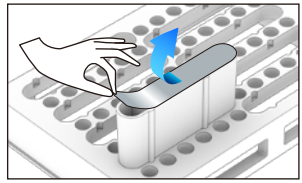


5. Close the front door of the instrument.

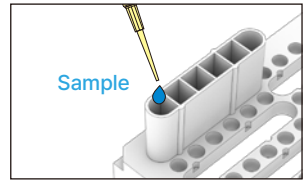
C-2. DNA extraction procedure (W7122, W7123)



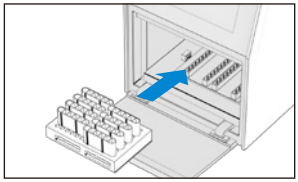
1. Mount the 6-well Strip onto the Strip Adapter Plate.



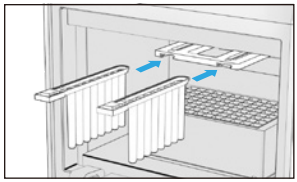
2. Carefully peel off the film of the 6-well Strip not to cross-contaminate.



3. Add 300 µL of the sample 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** ▶ **Fecal 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.

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				

TROUBLE SHOOTING GUIDE

Problem	Possible causes	Recommendations
Low yield	Too much starting materials	Too much starting materials may bring about inefficient lysis, followed by poor DNA yields. Keep the maximum weight of starting material as described on procedure.
	Too old or improperly stored sample used	DNA can be degraded, especially when the tissues are too old or improperly stored. Use a fresh sample.
	Insufficient disruption	Pulverizing of the sample is a critical step for good result. Incompletely disrupted sample will result in poor lysis, followed by poor yield. Thoroughly pulverize the tissue to get a fine powder whenever possible.
Low purity	Insufficient lysis	Too much starting material can lead to poor lysis, followed by low purity of DNA. Use a lesser sample.
	Co-transfer of debris	When transferring the sample mixture into the sample well, be careful not to co-transfer the debris of pellet. This will decrease the purity of DNA.
Degraded DNA	Too old or improperly stored sample used	DNA can be degraded, especially when the tissues are too old or improperly stored. Use a fresh sample.
	Excessive or retarded shredding	Good results need to pulverize the sample thoroughly. However, excessive or retarded shredding of samples will lead to damage to DNA.
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.

ORDERING INFORMATION

Product	Cat No.	Package	Note
WizMag™ Fecal DNA	W7120	64 Prep	16 prep/run
	W7121	192 Prep	
	W7122	32 Prep	Single prep
	W7123	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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