RUO

WizMag™ Seed DNA

User Manual

Ver 2.0

REF W7050 | W7051 | W7052 | W7053

For Research Use Only

wizbiosolutions
THE INNOVATION OF GENOMICS

INTENDED USE

The WizMag™ Seed DNA kit is designed to be used on the CLEO™ AP16 Nucleic Acid Extractor System and provides a fast and easy extraction method of total DNA from various plant seeds and bulbs. Purified DNA is free of enzyme inhibitors such as polysaccharides and polyphenolics, and highly suited for downstream applications such as PCR-based or enzyme-based reactions.

KIT CONTENTS

Contents	W7050	W7051	W7052	W7053	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 BLB	40 mL	110 mL	20 mL	55 mL	
Buffer SLB	40 mL	110 mL	20 mL	55 mL	
Buffer PKR	1 mL	3 mL	1 mL	1 mL	
Proteinase K*	14 mg	14 mg x 2	14 mg	14 mg	
RNase A solution*	340 µL	1 mL	170 µL	500 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.
* After dissolving, Proteinase K solution and RNase A solution should be stored at 2 ~ 8°C for optimal conservation.

QUALITY CONTROL ANALYSIS

In accordance with Wizbiosolutions Inc. ISO 13485-certified Quality Management System, each lot WizMag™ Seed 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.

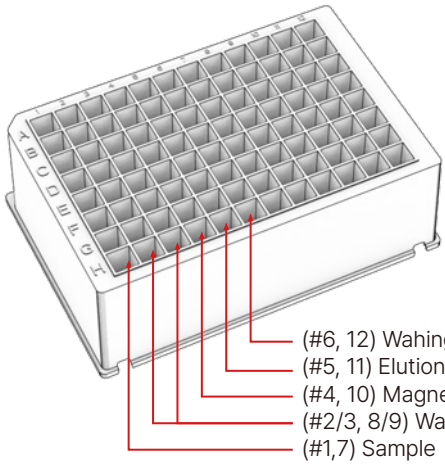
PRECAUTIONS

- 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 (W7050 | W7051)

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 (W7052 | W7053)

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

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	8
Well #	4	1	2	3	4	6	5	4
Step	Beads	Bind	Wash	Wash	Wash	Wash	Elute	Discard
Wait time	-	-	-	-	-	-	03:00	-
Mix time	00:20	10:00	02:00	02:00	02:00	02:00	05:00	00:20
Collect time	00:25	00:30	00:25	00:25	00:25	00:25	00:45	-
Volume(μL)	750	900	750	750	750	750	100	750
Mixing speed	Medium	Fast	Fast	Fast	Fast	Fast	Bottom	Medium
Collect speed	Strong	Strong	Strong	Strong	Strong	Strong	Strong	Normal
Temperature		Off					60°C	

B. Sample Preparation

- Prepare 1.5 mL or 2.0 mL microcentrifuge tube.
- Prepare 65°C water bath or dry bath.

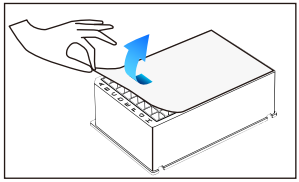
1. **Grind seeds or bulbs sample into a fine powder.**
   - This step is critical for good results. Pulverization should be carried out quickly and completely.
   - A mortar and pestle with liquid nitrogen is a good conventional method for pulverizing, but other methods like a bead-beating instrument or a rotor-stator homogenizer can be good alternatives. Follow the instruction manuals for those methods.
2. **Place up to 100 mg of seeds or up to 200 mg of bulbs into a 1.5 mL or 2 mL tube.**
   - Use up to 50 mg per prep if the sample is flax/alfalfa seeds or dried bulbs.
   - Good results need to use the sample freshly and thoroughly pulverized.
3. **Apply 500 μL of SLB (for seeds or dried bulbs) or BLB (for bulbs) and 5μL of RNase A solution into the tube. Vortex to get homogenate.**

Sample	Bulbs	Dried bulbs	Seeds	Flaxseed/Alfalfa seed
Max. wt/p	200 mg	50 mg	100 mg	50 mg
Lysis buffer	BLB	SLB	SLB	SLB

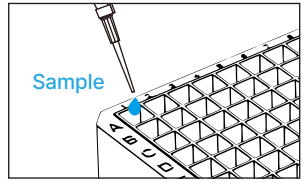
4. **Apply 10 μL of Proteinase K solution into the tube and vortex for 15 seconds to mix completely.**
   - Mix completely to make the lysate homogenate without any clumps.
5. **Incubate for 15 minutes at 65°C.**
   - Occasional vortex during incubation may accelerate the lysis. The use of a specialized instrument such as a thermo-mixer will accelerate the lysis.

6. **Centrifuge for 3 minutes at 13,000 xg or full speed.**
   - When the sample contains a lot of low-density components such as lipids, a film-like layer may be formed on the surface after centrifugation. In this case, carefully pipet the cleared lysate of the intermediate layer so as not to co-transfer as much debris as possible.
7. **Use the 300 µL of cleared supernatant as a sample.**

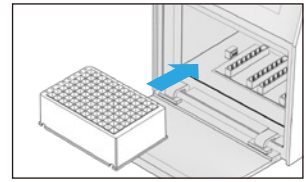
C-1. DNA extraction procedure (W7050, W7051)



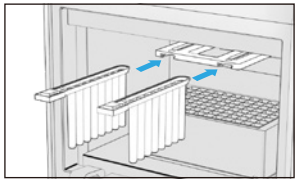
1. Carefully peel off the film of the 96-well Plate not to cross-contaminate.



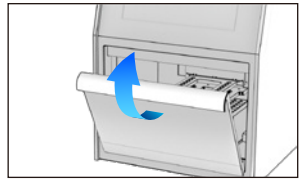
2. Add 300 µL of the sample into the each first well (#1,7)



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

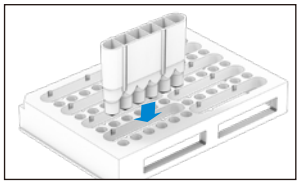


4. Insert a Plunger all the way into the socket above the 96-well Plate.

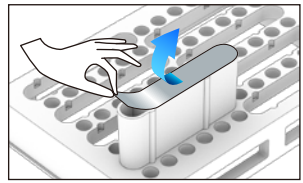


5. Close the front door of the instrument.

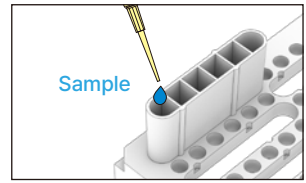
C-2. DNA extraction procedure (W7052, W7053)



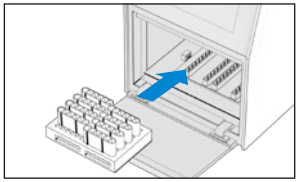
1. Mount the 6-well Strip onto the Strip Adapter Plate.



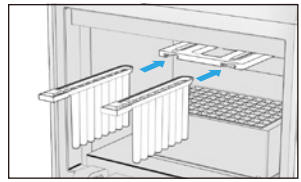
2. Carefully peel off the film of the 6-well Strip not to cross-contaminate.



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** ▶ **Seed 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 70 - 90 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
	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™ Seed DNA	W7050	64 Prep	16 prep/run
	W7051	192 Prep	
	W7052	32 Prep	Single prep
	W7053	96 Prep	
CLEO™ AP16 Nucleic acid Extractor	CL2016	1 system	1-16 sample
CLEO™ AP48 Nucleic acid Extractor	CL2048	1 system	1-48 sample

wizbiosolutions
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Technical Support

www.wizbiosolution.com
support@wizbiosolution.com
+82 70 7603 5066

Wizbiosolutions Inc.
#1103, 1405, 1406, A-dong, 14, Sagimakgol-ro 45beon-gil,
Jungwon-gu, Seongnam-si, Gyeonggi-do, Republic of Korea
B237~242, 14, Galmachi-ro 288beon-gil, Jungwon-gu,
Seongnam-si, Gyeonggi-do, Republic of Korea

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