RUO

WizMag™ Plant RNA plus

User Manual

Ver 2.0

REF W7084 | W7085 | W7086 | W7087

For Research Use Only

wizbiosolutions
THE INNOVATION OF GENOMICS

INTENDED USE

The WizMag™ Plant RNA plus kit is designed to be used on the CLEO™ AP16 Nucleic Acid Extractor Systemand provides a fast and easy purification of total RNA from various plant tissues including leaves, stems, roots, and other plant tissues. This kit provides the optimized method which is effective for isolating intact plant RNA without contaminating plant secondary metabolites, such as polyphenols and polysaccharides. Purified RNA is highly suited for downstream applications such as PCR-based or enzyme-based reactions.

KIT CONTENTS

Contents	W7084	W7085	W7086	W7087	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 MPL*	40 mL	120 mL	20 mL	55 mL	
Buffer DRB	500 µL	1.3 mL	250 µL	700 µL	
0.25 M EDTA solution	200 µL	400 µL	200 µL	200 µL	
Buffer DSB	500 µL	500 µL	500 µL	500 µL	
DNase I (lyophilized)**	200 U	600 U	100 U	300 U	
Blank solution N	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, lyophilized DNase I should be stored at 2 - 8°C for optimal conservation. Long exposure to heat sources can deteriorate the performance of the kit significantly.

* During shipment or storage under cold ambient conditions, a precipitate can be formed in buffer MPL. Heat the bottle at 20°C ~40°C to dissolve completely before use.

** After reconstitution, DNase I solution should be stored at -20°C for optimal conservation of activity.

QUALITY CONTROL ANALYSIS

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

RECONSTITUTION OF DNASE I

Before the experiment, lyophilized DNase I should be reconstituted in Buffer DSB. To obtain a working solution (2 U/µL), add 100 µL (600 µL) of Buffer DSB to the tube containing a lyophilized DNase I. Do NOT vortex while dissolving. Store the reconstituted DNase I solution at -20°C.

PREVENTING RNASE CONTAMINATION

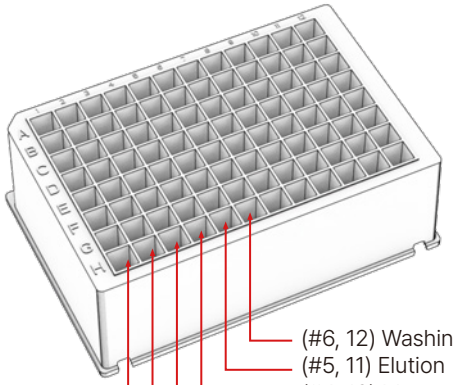
RNase can be introduced accidentally during RNA preparation. Various factors such as reagents, air, dust, and human hands or skin, can be the sources of RNase contamination. Always wear disposable gloves and use sterile, disposable plastic wares. Use the pipette reserved for RNA work.

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.
- RNase can be introduced accidentally during RNA preparation. Various factors such as reagents, air, dust, and human hands or skin, can be the sources of RNase contamination. Always wear disposable gloves and use sterile, disposable plastic wares. Use the pipette reserved for RNA work. Maintain a separate area for RNA work. Carefully clean the surfaces.

COMPOSITION OF THE PRE-PACKED 96-WELL PLATE (W7080 | W7081)

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 (W7082 | W7084)

(#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:

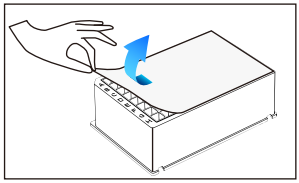
No.	1	2	3	4	5	6	7	8
Well No.	4	1	2	3	4	6	5	4
Step	Bead	Bind	Wash	Wash	Wash	Wash	Elute	Discard
Wait time	-	-	-	-	-	-	03:00	-
Mix time	00:20	10:00	02:00	01:00	01:00	01:00	05:00	00:20
Collect time	00:25	00:30	00:25	00:25	00:25	00:25	00:30	-
Volume(µL)	750	800	750	750	750	750	100	750
Mixing speed	Medium	Fast	Fast	Fast	Fast	Fast	Bottom	Medium
Collect speed	Strong	Strong	Strong	Strong	Strong	Strong	Strong	Normal
Temperature		Off					Off	

B. Sample Preparation

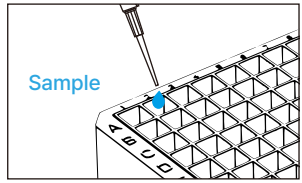
1. **Place up to 100 mg (wet) or 25 mg (dried) of ground plant tissue into a 1.5 mL or 2 mL tube.**
   - The sample must be handled quickly under low temperatures for the recovery of intact RNA.
   - Grind fresh or frozen plant tissue to a fine powder quickly and completely. Coarse particles due to incomplete pulverization will make the yield and quality of RNA poor.
   - A mortar and pestle 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. **Add 500 µL of Buffer MPL into the tube.**
3. **Vortex vigorously for 15 seconds and incubate for 5 minutes at room temperature.**
   - Mix completely to make the lysate homogenate.
4. **Centrifuge for 5 mins at 14,000 xg or full speed.**
   - The sample must be handled quickly under low temperatures for the recovery of intact RNA.

5. **Use the 400 µL of cleared supernatant (sample lysate) as a sample.**
   - Coarse sample particles due to incomplete pulverization will make difficult the separation of the debris and the supernatant. In this case, the use of the 'Wide-bore tip' will be help take the soup.

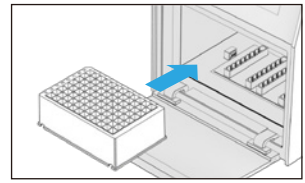
C-1. RNA extraction procedure (W7084, W7085)



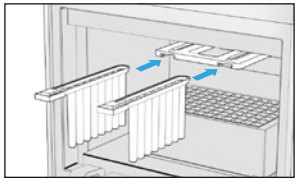
1. Carefully peel off the film of the 96-well Plate not to cross-contaminate.



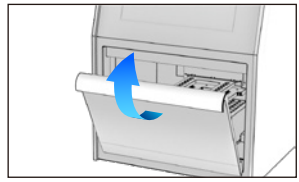
2. Add 400 µL of the sample lysate into the each first well (#1, 7)



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

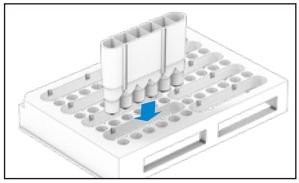


4. Insert a Plunger all the way into the socket above the 96-well Plate.

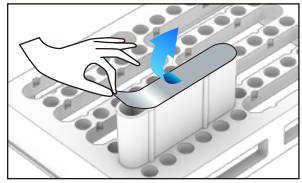


5. Close the front door of the instrument.

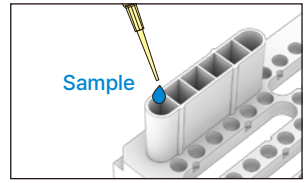
C-2. RNA extraction procedure (W7086, W7087)



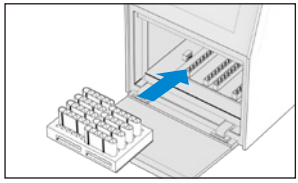
1. Mount the 6-well Strip onto the Strip Adapter Plate.



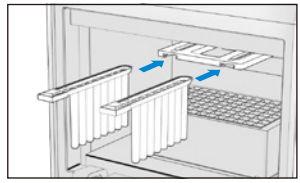
2. Carefully peel off the film of the 6-well Strip not to cross-contaminate.



3. Add 400 µL of the sample lysate 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 Plate.

6. Close the front door of the instrument.
7. Select **MENU** ▶ **DNA** ▶ **Plant RNA** 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 RNA 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.

D. DNA removal

* Prepare 75°C water bath or heat block.

1. 1. Freshly prepare the reaction mixture in a microtube as below:
   - 50 µL RNA eluate
   - 5 µL Buffer DRB
   - 1 µL DNase 1 solution (2 U/µL)
2. Incubate the mixture for 10 minutes at room temperature.
3. Add 1 µL of 0.25 M EDTA solution.
4. Inactivate DNase 1 by incubating at 75°C for 10 minutes.
   - RNA can be deteriorated by exposure to high temperatures. If the quality of RNA is essential, it is recommended to purify RNA with other commercial RNA clean-up kits.

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

TROUBLE SHOOTING GUIDE

Problem	Possible causes	Recommendations
Low recovery of RNA	Too much starting materials	Too much starting materials will bring about inefficient lysis, followed by poor RNA yields. Keep the maximum amount of starting material asdescribed in the procedure.
	Poor quality of starting material	Use a freshly harvested sample if possible. Sample should always be handled quickly under low temperature.
	Insufficient disruption	Pulverizing the sample is a critical step for good results. Improperly disrupted samples will result in poor lysis, followed by poor yield. Pulverize quickly and completely the tissue under liquid nitrogen.
	The lysate not homogenized thoroughly	It is important for good results to make the lysate homogenized after the addition of buffer MPL.
RNA degraded	Improper storage of starting materials	The starting sample should be quickly treated under low temperatures. Long exposure to high temperatures or retarded processing would be a cause of degradation.
	Poor quality of starting material	Harvested plant tissue should be stored under -80°C for later use. RNA will be gradually degraded even at -20°C. Use a freshly harvested sample if possible.
	RNase contamination	RNase can be introduced accidentally into a preparation at any step. Always wear disposable gloves and use RNase-free plasticwares. Do not use shared equipment if possible.
DNA contamination	Large DNA mass of starting materials	Some plant tissues may have a larger mass of DNA than others. In this case, it is recommended to reduce the starting amount or perform the optional DNase I treatment. Shorter incubating times after the addition of MPL can be helpful to prevent DNA-contamination.
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 recommended to shake off the deposit on the film with holding the plate or the tube tightly.

ORDERING INFORMATION

Product	Cat No.	Package	Note
WizMag™ Plant RNA plus	W7084	64 Prep	16 prep/run
	W7085	192 Prep	
	W7086	32 Prep	Single prep
	W7087	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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