RUO

WizMag™ Cell/Tissue RNA

User Manual

Ver 2.0

REF W7090 | W7091 | W7092 | W7093

For Research Use Only

INTENDED USE

The WizMag™ Cell/Tissue RNA kit is designed to be used on the CLEO™ AP16 Nucleic Acid Extractor System. This kit provides a fast and easy method for the purification of total RNA from various biological samples including animal tissues and cultured cell. This kit utilizes advanced magnetic-silica technology to purify high-quality total RNA. Co-purified DNA can be easily removed by DNase I. Purified RNA is highly suited for downstream applications such as PCR-based or enzyme-based reactions.

KIT CONTENTS

Contents	W7090	W7091	W7092	W7093	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 RTL *	32 mL	95 mL	16 mL	48 mL	
Blank solution N	500 uL	500 uL	500 uL	500 uL	

This kit is delivered under ambient condition. Long exposure to heat source can deteriorate the performance of kit significantly.

* During shipment or storage under cold ambient condition, a precipitate can be formed in Buffer RTL. 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™ Cell/Tissue RNA kit is tested against predetermined specifications to ensure consistent product quality.

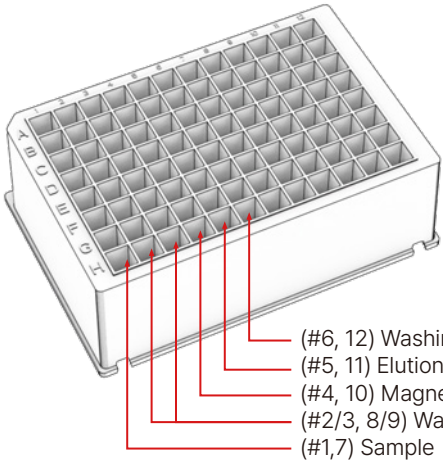
PRECAUTIONS

- This product is reserved exclusively 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 (W7090 | W7091)

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 (W7092 | W7093)

(#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	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	08:00	01:00	01:00	00:45	00:45	03:00	00:20
Collect time	00:30	00:30	00:25	00:25	00:25	00:25	00:30	-
Volume(μL)	500	800	750	750	750	500	80	750
Mixing speed	Medium	Fast	Fast	Fast	Fast	Fast	Bottom	Medium
Collect speed	Strong	Strong	Strong	Strong	Strong	Strong	Strong	Normal
Temperature		Off					Off	

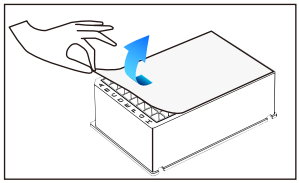
B. Sample Preparation

- Prepare 1.5 mL or 2.0 mL microcentrifuge tube
- Prepare ß-mercaptoethanol (CAS No. 60-24-2)

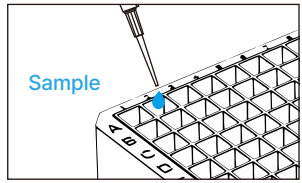
1. **Cultured cells**
   a. Harvest up to 5 x 10^6 cells into a 1.5 mL or 2.0 mL microcentrifuge tube.
      - Discard the culture media as much as possible.
   b. Resuspend the cell pellet in 400 μL of Buffer RTL by vortexing or pipetting.
      - For efficient resuspending, it is helpful to loosen and disrupt the pellets by flickering or vortexing before the addition of Buffer RTL.
      - It is critical to make the lysate homogenate for good results.
   c. Use 400 μL of the lysate as a sample.

2. **Animal tissues**
   a. Place up to 30 mg of a finely ground tissue into a 1.5 mL or 2 mL tube.
      - The sample must be disrupted quickly and completely under low temperatures for the recovery of intact RNA.
      - 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.
      - Tissue samples can be disrupted in Buffer RTL.
   b. Add 450 μL of Buffer RTL and 10 μL of β-mercaptoethanol into the tube.
      - β-mercaptoethanol can be freshly added into Buffer RTL before use.
   c. Mix completely to make the mixture homogenate by vigorous vortexing or pipetting.
   d. Incubate for 3 minutes at room temperature and centrifuge for 1 minutes at 8,000xg or above.
      - No need to centrifuge if any debris does not exist in the mixture.
   e. Use 400 μL of the cleared lysate as a sample.

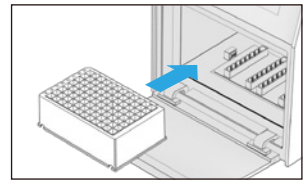
C-1. RNA extraction procedure (W7090, W7091)



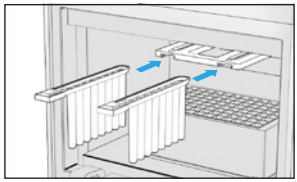
1. Carefully peel off the film of the 96-well Plate not to cross-contaminate.



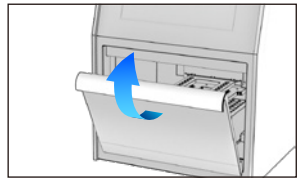
2. Add 400 µL of the sample lysate into the each first well (#1, 7)



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

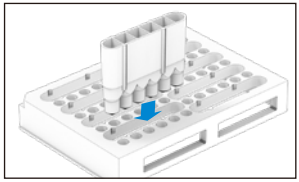


4. Insert a Plunger all the way into the socket above the 96-well Plate.

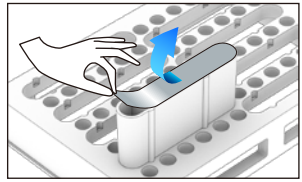


5. Close the front door of the instrument.

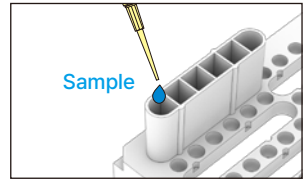
C-2. RNA extraction procedure (W7092, W7093)



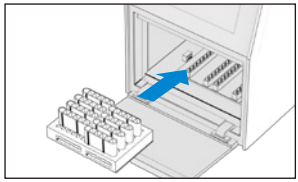
1. Mount the 6-well Strip onto the Strip Adapter Plate.



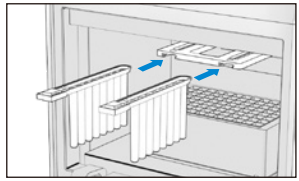
2. Carefully peel off the film of the 6-well Strip not to cross-contaminate.



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** ▶ **Cell/Tissue 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 50 - 70 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.

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™ Cell/Tissue RNA	W7090	64 Prep	16 prep/run
	W7091	192 Prep	
	W7092	32 Prep	Single prep
	W7093	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 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.
	The lysate is not homogenized thoroughly	It is important for good results to make the lysate homogenized after the addition of Buffer RTL.
RNA degraded	Inappropriate handling 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.
	Improper storage of starting materials	Harvested tissues 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	DNase not treated	This kit is designed to prepare RNA, but DNA can be co-purified depending on the sample. To remove DNA from the eluate, treat the eluate with DNase I.
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.

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