RUO

WizMag™ Bacterial RNA

User Manual

Ver 1.0

REF W7220 | W7221 | W7222 | W7223

For Research Use Only

wizbiosolutions
THE INNOVATION OF GENOMICS

INTENDED USE

The WizMag™ Bacterial RNA kit is designed to be used on the CLEO™ AP16 & AP48 nucleic acid extractor system. It provides an automated method for purifying total RNA from various cell samples, including bacterial cell cultures and yeast cell cultures. The purified RNA is free of enzyme inhibitors and other contaminants, making it highly suitable for downstream applications such as PCR-based or enzyme-based reactions.

KIT CONTENTS

Contents	W7220	W7221	W7222	W7223	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™ B1 tube	64 ea	192 ea	32 ea	96 ea	
Buffer BRS	35 mL	100 mL	20 mL	55 mL	
Blank solution N	500 µL	500 µL	500 µL	500 µL	

This kit is delivered under ambient conditions. If used immediately upon arrival, all components can be stored at room temperature (15-25°C)

QUALITY CONTROL ANALYSIS

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

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. Maintain a separate area for RNA work. Carefully clean the surfaces.

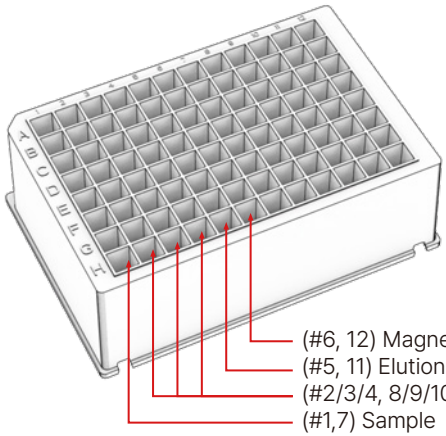
PRECAUTIONS

- 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 (W7220 | W7221)

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 (W7222 | W7223)

(#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 No.	6	1	2	3	4	5	6
Step	Beads	Lysis	Wash	Wash	Wash	Elute	Discard
Wait time	-	-	-	-	-	03:00	-
Mix time	00:20	10:00	03:00	02:00	02:00	03:00	00:20
Collect time	00:25	00:30	00:25	00:25	00:25	00:30	-
Volume(µL)	200	700	500	500	500	80	200
Mixing speed	Medium	Fast	Fast	Fast	Fast	Bottom	Medium
Collect speed	Strong	Strong	Strong	Strong	Strong	Strong	Normal
Temperature		Off				Off	

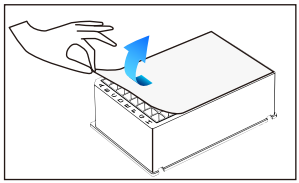
B. Sample Preparation

- Prepare β-mercaptoethanol or 1M DTT solution.

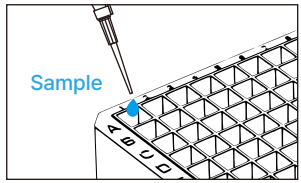
1. **Harvest up to 2 x 10^9 bacterial cultured cells or up to 10^8 yeast cultured cells in a 1.5ml tube by centrifugation and discard as much of the supernatant as possible.**
   - For Gram-negative bacteria, bypass the procedure below and follow these steps: add 20 µL of BME, resuspend the pellet in 200 µL of Buffer BRS, vortex to mix, incubate for 3 minutes at room temperature, and use the entire mixture as a sample..
2. **Add 20 µL of β-mercaptoethanol (BME) into the tube, resuspend the cell pellet thoroughly in 450 µL of Buffer BRS, and transfer all of the resuspension into a APowerbead tube.**
   - Alternatively, 10 µL of 1M DTT(1,4-Dithiothreitol) solution can be used instead of β -mercaptoethanol.
3. **Secure the Powerbead™ tube on the tube-holder adapter of the vortex machine or the bead-beating machine.**
   - The Powerbead™ tube should be mounted horizontally on the vortex machine.
4. **Vortex the tube on the highest setting (>2,000 rpm) for 8 minutes.**
   - Disrupting for too short a time may result in low yield, whereas disrupting for too long a time may cause RNA damage.
   - The 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 time may be as little as 1 minute when using high-speed cell-disrupting machines (e.g., GeneReady, FasPrep-24, Precellys 24, PowerLyzer 24) or as long as 10 minutes when using lower speeds (e.g., standard benchtop vortexer). See manufacturer's instruction for operating information.
5. **Let the Powerbead™ tube stand for 3 minutes to settle.**
   - Do not centrifuge the Powerbead™ tube. This will cause a decrease in RNA yield.

6. **Carefully pipette 200 µL of the supernatant and use it as a sample.**
   - Trace amounts of beads may be co-transferred during pipetting, but this will not significantly affect the result.

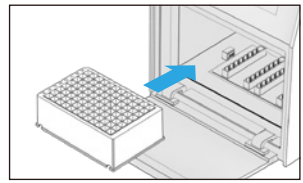
C-1. RNA extraction procedure (W7220, W7221)



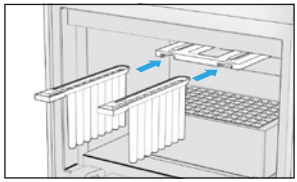
1. Carefully peel off the film of the 96-well Plate not to cross-contaminate.



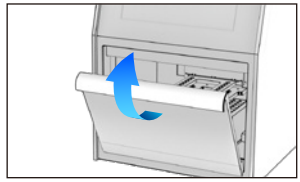
2. Add 200 µL of the sample into the each first well (#1,7)



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

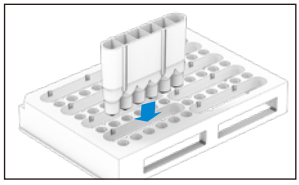


4. Insert a Plunger all the way into the socket above the 96-well Plate.

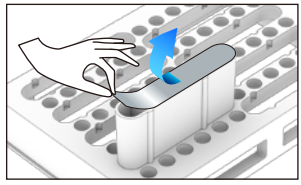


5. Close the front door of the instrument.

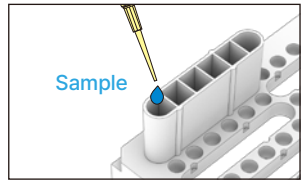
C-2. RNA extraction procedure (W7222, W7223)



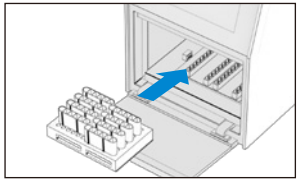
1. Mount the 6-well Strip onto the Strip Adapter Plate.



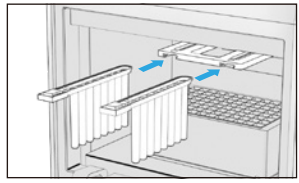
2. Carefully peel off the film of the 6-well Strip not to cross-contaminate.



3. Add 200 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** ▶ **RNA** ▶ **Bacterial 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 60 - 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.
    - Samples with a high cellulose content can lead to cloudy eluate, so proceed again with 50 mg of sample in this case.

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™ Bacterial RNA	W7220	64 Prep	16 prep/run
	W7221	192 Prep	
	W7222	32 Prep	Single prep
	W7223	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 critical step for good result. Incompletely disrupted sample 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 result to make the lysate homogenized after addition of Buffer WR1. Mix the lysate thoroughly.
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.
	Poor quality of starting material	Harvested wood 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 tissue samples may have a larger mass of DNA than others. In this case, reducing the starting amount or performing the optional DNase I treatment is recommended. Shorter incubating times after the addition of Buffer WR1 can help prevent DNA contamination.
Cloudy eluate	Cellulose not removed completely	Wood tissue consists primarily of cellulose complexes, with some samples containing particularly high amounts. Use half the weight for this 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 recommended to shake off the deposit on the film with holding the plate or the tube tightly.

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