

WizMag™ Fungal DNA

User Manual

Ver 1.0

REF W7180 | W7181 | W7182 | W7183

For Research Use Only



INTENDED USE

The WizMag™ Fungal DNA kit is designed to be used on the CLEO™ AP16 Nucleic Acid Extractor System for simple and easy purification of total DNA from fungal spores, mycelium, and yeast cells. This kit utilizes the silica beads for the disruption of fungal cell walls which are tough to lyse, and the bead beating can be performed on any vortex that has a 2 mL tube holder. 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 | W7180 | W7181 | W7182 | W7183 | Storage |
|--------------------------|---------|---------|--------|---------|-------------|
| No. of preparation | 64 | 192 | 32 | 96 | |
| 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™ Y1 tube | 64 ea | 192 ea | 32 ea | 96 ea | Room |
| Buffer YM1* | 30 mL | 90 mL | 16 mL | 45 mL | Temperature |
| Buffer YM2 | 5 mL | 12 mL | 3 mL | 7 mL | (15-25°C) |
| Buffer YM3 | 10.8 mL | 31.5 mL | 6.3 mL | 16.2 mL | |
| Enhancing solution P | 1.2 mL | 3.5 mL | 0.7 mL | 1.8 mL | |
| RNase A solution* | 120 µL | 360 µL | 64 µL | 180 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.

QUALITY CONTROL ANALYSIS

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

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.

^{*} Before first use, add all of RNase A solution into Buffer YM1, and store at 4°C.

- 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.

CHECK BEFORE FIRST USE

Preparation of Buffer YM1 containing RNase A

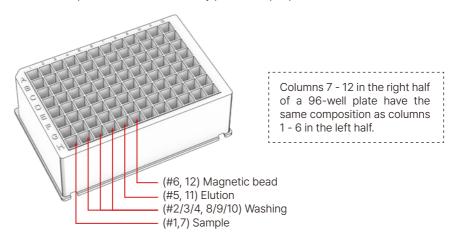
Before first use, add all of RNase A solution into Buffer YM1 and mix well by gentle swirling. Store RNase-containing Buffer YM1 at $2 \sim 8$ °C for conservation of activity.

Preparation of Buffer YM3 with enhancing solution P

Before first use, add all of the enhancing solution P into Buffer YM3 and mix well by gentle swirling. Store the Buffer YM3 at room temperature (20 - 25 °C).

COMPOSITION OF THE PRE-PACKED 96-WELL PLATE (W7180 | W7181)

A total of 16 samples can be simultaneously processed per plate.



COMPOSITION OF THE PRE-PACKED 6-WELL STRIP (W7182 | W7183)



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

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PROTOCOL

A. Setup of program (For CLEO™ AP16 & AP48 devices, preset program can be used.) Edit and run the experiment program as follows:

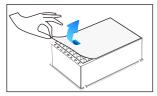
| No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---------------|--------|--------|--------|--------|--------|--------|---------|
| Well# | 6 | 1 | 2 | 3 | 4 | 5 | 6 |
| Step | Beads | Bind | Wash | Wash | Wash | Elute | Discard |
| Wait time | - | - | - | - | - | 03:00 | - |
| Mix time | 00:20 | 10:00 | 02:00 | 01:00 | 01:00 | 05:00 | 00:15 |
| Collect time | 00:25 | 00:30 | 00:25 | 00:25 | 00:25 | 00:30 | - |
| Volume(µL) | 200 | 800 | 750 | 750 | 750 | 100 | 200 |
| Mixing speed | Medium | Fast | Fast | Fast | Fast | Bottom | Medium |
| Collect speed | Strong | Strong | Strong | Strong | Strong | Strong | Normal |
| Temperature | | Off | | | | 60°C | |

B. Sample Preparation

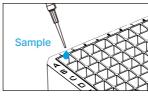
- Prepare 1.5 mL microcentrifuge tube and 65°C water bath or dry bath.
- Before first use, add all of RNase A solution into Buffer YM1, and store the Buffer YM1 at 4°C
- 1. Harvest 50 100 mg (wet weight) of fungal cells or $0.5 \sim 1 \text{ mL}$ of yeast cell culture (up to 10^8 cells) in a 1.5 mL microcentrifuge tube by centrifugation and discard the supernatant as much as possible.
- 2. Resuspend the pellet thoroughly in 400 µL of Buffer YM1.
 - For efficient resuspending, it is helpful to loosen and disrupt the pellets by flickering or vortexing before the addition of Buffer YM1.
 - · Check that RNase A solution has been added to Buffer YM1 before first use.
- Transfer all of the resuspension into the Powerbead™ Y1 tube and secure the tube on the tube-holder adapter of the vortex machine or bead-beating machine.
 - Powerbead[™] tube should be mounted horizontally on the vortex machine for better-disrupting efficiency.
- 4. Vortex the tube on the highest setting (>2,000 rpm) for 6 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 1 minute when using high-speed cell disrupting machines (e.g., GeneReadyTM, FastPrep-24TM PrecellysTM 24, Power-LyzerTM 24) or as long as 15 minutes when using lower speeds. See the manufacturer's literature for operating information.
- 5. Apply 50 µL of Buffer YM2 into the tube, vortex to mix thoroughly, and incubate the tube at 65°C for 10 minutes.
- 6. Cool the tube to room temperature, add 150 µL of Buffer YM3, and vortex to mix thoroughly.
 - If the tube is not cooled to room temperature, the efficiency of precipitating may decrease, followed by poor DNA quality.
 - Check that the enhancing solution P has been added to Buffer YM3 before first use.

7. Centrifuge at >13,000 xg for 3 minutes and use 300 μL of the supernatant as a sample.

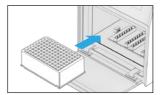
C-1. DNA extraction procedure (W7180, W7181)



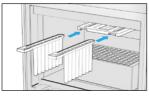
1. Carefully peel off the film of the 96-well Plate not to cross-contaminate.



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 (W7182, W7183)



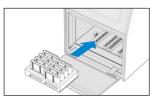
1. Mount the 6-well Strip onto the Strip Adapter Plate.



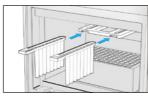
Carefully peel off the film of the 6-well Strip not to cross-contaminate.



3. Add 300 µL of the sample into the each first well (#1)



 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.

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- 6. Close the front door of the instrument.
- 7. Select MENU ▶ DNA ▶ Fungal 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.

TROUBLE SHOOTING GUIDE

| Problem | Possible causes | Recommendations |
|------------------------------|---|--|
| Low or no | Starting sample is not fresh or improperly stored | DNA yield may be reduced if the sample is improperly stored or outdated. Use a freshly harvested sample. |
| recovery | Low beating power | Low beating power can't properly disrupt the cell walls. Bead-beating should be carried out at maximum power on a conventional vortex machine. Please refer to the manufacturer's instruction manual for other disrupting machines. |
| 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. |
| | Too much sample amount used | Do not overload the sample. Keep the maximum sample weight or cell number in the sample as a procedure. |
| Degraded DNA | Too much power for bead-beating | Too high speed or longer time for bead-beating can cause the degradation of DNA. It is recommended to follow the basic procedure for the first time and then optimize the speed and the time for the sample at the next preparation. |
| | Starting sample is not fresh or improperly stored | DNA can be degraded if the sample is improperly stored or outdated. Use a freshly harvested sample if possible. |
| 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. |

SYMBOL GLOSSARY

| REF | Catalogue number | Ш | Manufacturer | Σ | Use-by date |
|----------|---------------------------------------|-----------|----------------------|----|-------------------------|
| LOT | Batch code | 2 | Do not re-use | 1 | Temperature limitation |
| RUO | Research use only | (i | Instructions for use | 漛 | Keep away from sunlight |
| Σ | Contents sufficient for <n> tests</n> | <u>(i</u> | Caution | ** | Keep dry |
| ® | Do not use if package is damaged | | | | |

ORDERING INFORMATION

| Product | Cat No. | Package | Note | |
|-----------------------------------|---------|----------|---------------|--|
| | W7180 | 64 Prep | 16 prep/run | |
| WizMag™ Fungal DNA | W7181 | 192 Prep | то ргер/гип | |
| | W7182 | 32 Prep | - Single prep | |
| | W7183 | 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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Technical Support





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