EIV32024E

ExCell Bio

ResiQuant® Mycoplasma DNA Isolation Kit

For Research and Manufacturing Use

Not Intended for Diagnostic and Therapeutic Use

User Manual

Catalog Number	CRB00-0031S
	CRB00-0031
	CRB00-0032

PRODUCT DESCRIPTION

Mycoplasma possesses inherent potential for infectious risks, capable of infecting humans, animals, and plants, including cell cultures employed in the production of bio-products and pharmaceuticals. Infection by mycoplasma leads to alterations in host cell DNA replication, RNA transcription, and protein expression, modifications in membrane antigens, thereby affecting cell growth and proliferation. The cell culture and indicator cell culture assays are conventional methods for mycoplasma test. They are either time-consuming or less sensitive, unable to meet the rapid release requirement for those biopharmaceuticals like therapeutic cell drugs with short shelf-life. Some Pharmacopeias, including EP, USP, and JP, recommend using nucleic acid amplification testing (NAT) method as a complementary or alternative method for mycoplasma release test.

ResiQuant® Mycoplasma DNA Isolation Kit is designed to work together with ResiQuant® Mycoplasma Detection Kit (Taqman) (CAT. CRB00-1011/CRB00-1012) in the rapid mycoplasma release test. Including the species required in the EP 2.6.7, the assay is designed to detect a broad range of species. According to the suggestion in EP 2.6.7, ResiQuant® Mycoplasma Detection Kit (Taqman) (CAT. CRB00-1011/CRB00-1012) have been validated regarding to specificity, detection limit and robustness. The parallel comparability study demonstrated ResiQuant® Mycoplasma Detection Kit (Taqman) (CAT. CRB00-1011/CRB00-1012) reach the LOD of 10 CFU/mL, compatible to the sensitivity of cell culture method. ResiQuant® Mycoplasma DNA Isolation Kit optimized the whole process of DNA extraction, gives corresponding suggestions to different types of complicated sample matrix, enabling the isolation of trace amounts of mycoplasma DNA from variable biological samples.

For the first-time use, it is recommended to confirm the suitability of this method to the intended sample matrix, ruling out possible matrix interferences effects. Please note that the test results only reflect the status of the samples. The sampling method has important impact on the result. The risk is suggested be carefully evaluated based on the test and sampling strategy together. The official guidances or pharmacopeias can be taken as references for starting test design. For example, the minimum starting sample volume could be set at 0.5-1 mL according to Chinese Pharmacopeia 2020. The optional strategies for different types of samples are given blow. Validation in case by case manner is suggested before kickoff.

PERFORMANCE, APPLICATION AND RESTRICTION

This kit is designed for extraction of mycoplasma nucleic acid from biological samples, including cell culture supernatant, bovine serum, serum-free culture medium, cell freezing solution, and banking or therapeutic cells. High cell density as 1E6 to 1E7 per milliliter is endurable in direct extraction manner.

SPECIFICATION, STORAGE AND TRANSPORTATION REQUIREMENT

Serial number	Component	CRB00-0031 (15T)	CRB00-0032 (30T)	CRB00-0031S (15T)
Box 1	***Myco* Lysis Buffer 1**	300 μL	300 μL × 2	300 μL
	Proteinase K Buffer	450 μL	450 μL × 2	450 μL
	5M NaCl	750 μL	750 μL × 2	750 μL
	***Myco* Lysis Buffer 2**	3 mL	6 mL	3 mL
	***Myco* Magnetic Beads**	225 μL	225 μL × 2	225 μL
	***Myco* Wash Buffer**	8 mL	15 mL	8 mL
	***Myco* Elution Buffer**	1.5 mL	1.5 mL × 2	1.5 mL
Box 2	**Proteinase K**	450 μL	450 μL × 2	450 μL

Storage: Store Box 1 at room temperature and Box 2 at -40℃ to -18℃.

Shelf Life: 12 months under specified conditions.

Transportation: Box 1 should be kept at room temperature, and Box 2 in dry ice.

EXPERIMENTAL PREPARATION

Instruments and Reagents Required but Not Included in the Kit

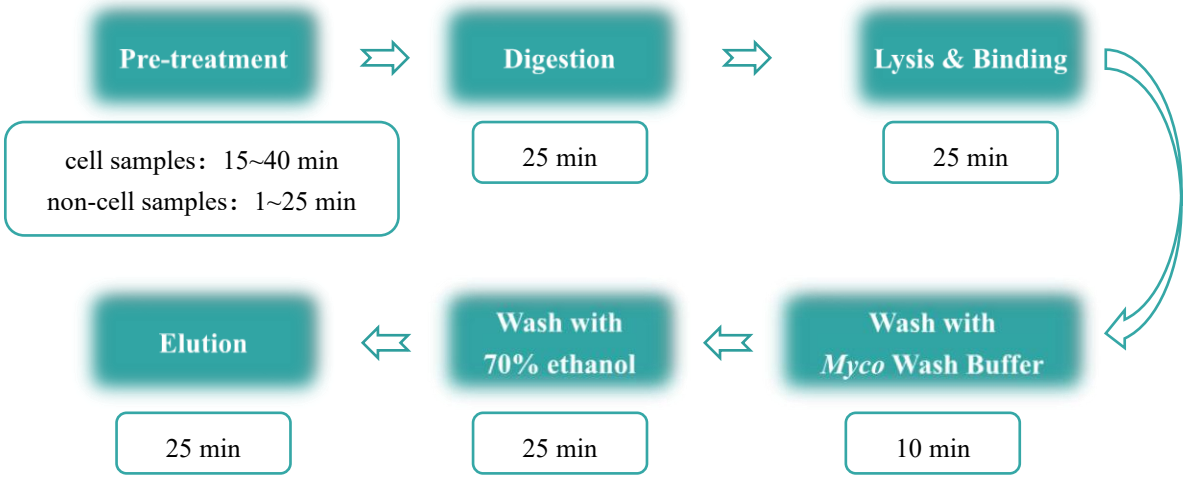
- High-speed centrifuge;
- Vortex oscillator;
- Magnetic rack;
- Mini centrifuge;
- Dry bath;
- 1.5 mL sterile low-adsorption centrifuge tube;
- Pipettes and corresponding sterile low-adsorption filter tips.
- Ethanol (AR);
- Isopropanol (AR);
- Sterile phosphate buffer saline（PBS）(0.15 M, pH 7.2);
- Sterile ultrapure water (PCR grade).

First-time Use

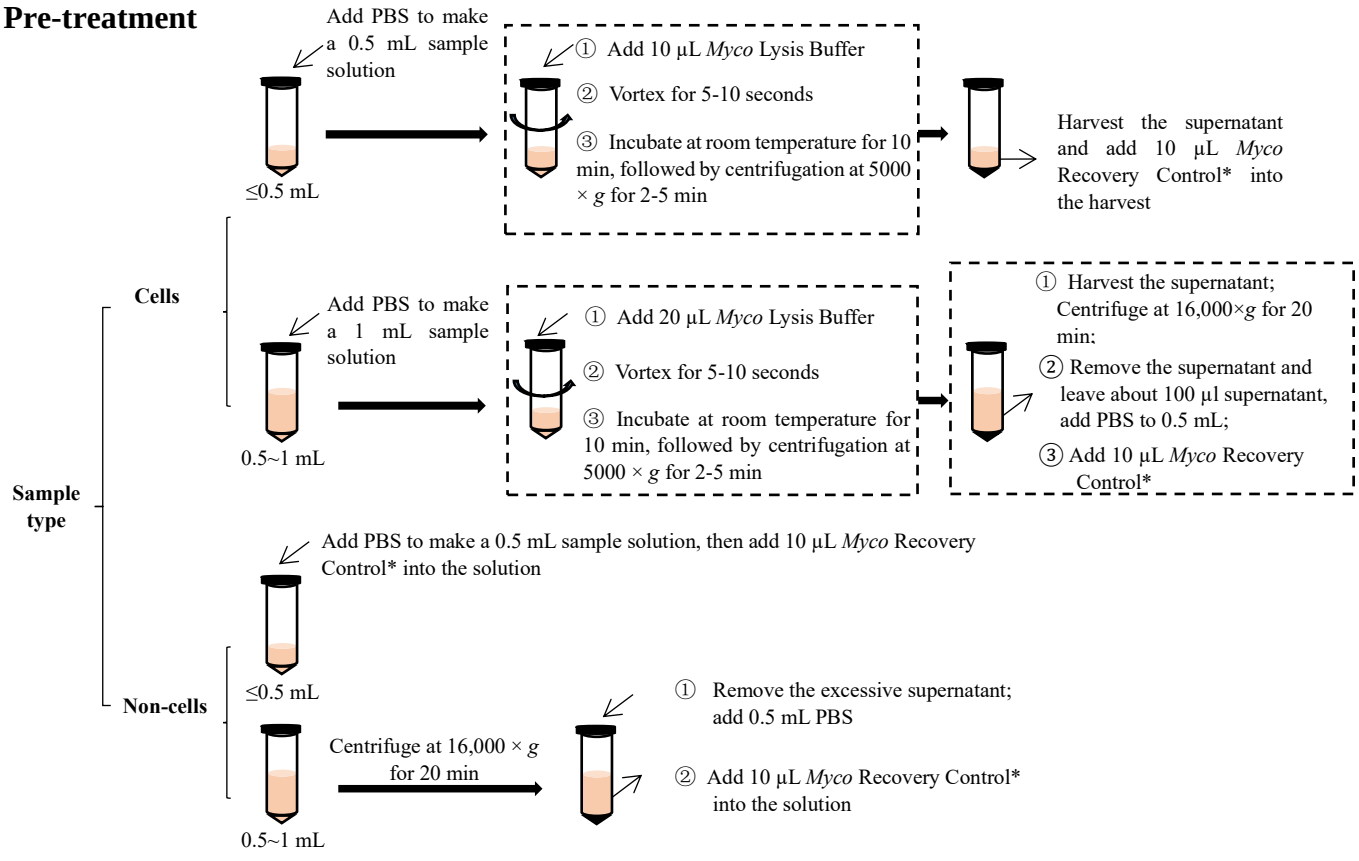
- In case of precipitation or crystal observed in following reagents like Proteinase K Buffer, 5M NaCl, *Myco* Lysis Buffer 2, or *Myco* Wash Buffer, incubate the certain vials at 37℃ for 5-10 minutes to dissolve the precipitate or crystals;
- Before use, add the appropriate volume of ethanol to the *Myco* Wash Buffer as indicated on the tube;
- Prepare freshly 70% ethanol with sterile ultrapure water. Keep the container well sealed to prevent ethanol evaporation.

EXPERIMENTAL PROCEDURE

Operational Flowchart



Pre-treatment



Note：

(1) * Myco Recovery Control (RC) is a component of ResiQuant® Mycoplasma Detection Kit (Taqman). It serves as a recovery control to monitor the process of mycoplasma DNA isolation. In prior to use, vortex the tube for 5-10 seconds is recommended;

(2) For variant sample volume, the centrifugation process condition needs to be validated in advance.

- **Preparation for Cell-type Samples**

A. For samples with a volume no more than 0.5 mL:

(1) Add PBS to make 0.5 mL sample solution;

(2) Add 10 µL of *Myco* Lysis Buffer 1 to the sample;

(3) Vortex the tube for 5-10 seconds to mix thoroughly;

(4) Incubate the tube at room temperature for 10 minutes;

(5) Centrifuge the tube at 5000 × g for 2-5 minutes;

(6) Carefully transfer the supernatant to a new tube without touching the precipitate;

(7) Add 10 µL of *Myco* Recovery Control to the harvest;

B. For samples with volume at 0.5 mL to 1 mL:

(1) Add PBS to make 1 mL sample solution;

(2) Add 20 µL of *Myco* Lysis Buffer 1 to the sample;

(3) Vortex the tube for 5-10 seconds to mix thoroughly;

(4) Incubate the tube at room temperature for 10 minutes;

(5) Centrifuge the tube at 5000 × g for 2-5 minutes;

(6) Carefully transfer the supernatant to a new tube without touching the precipitate；

(7) Centrifuge the sample at 16,000 × g for 20 minutes;

(8) Carefully keep the tip above the liquid surface to remove most of supernatant without touching the sediment. Please note that it is no need to remove all liquid. To leave behind about 100 µL can decrease the risk of unexpected target loss;

(9) Add PBS to make 0.5 mL sample solution;

(10) Add 10 µL of *Myco* Recovery Control to the harvest;

- **Preparation for Non-cell-type Samples**

A. For samples with a volume no more than 0.5 mL:

(1) Add PBS to make 0.5 mL sample solution;

(2) Add 10 µL of *Myco* Recovery Control to the sample;

B. For samples with a volume of 0.5 mL to 1 mL:

(1) Centrifuge the sample at 16,000 × g for 20 minutes;

(2) Carefully keep the tip above the liquid surface to remove most of supernatant without touching the precipitate. Please note that it is no need to remove all liquid. To leave behind about 100 µL can decrease the risk of unexpected target loss;

(3) Add PBS to make 0.5 mL sample solution;

(4) Add 10 µL of *Myco* Recovery Control to the sample solution;

- **Controls**

Negative Control Sample (NCS): Based on the sample matrix, prepare negative control solution and follow the same pre-treatment process to make a RC-spiked negative control solution;

Attention：

(1) Based on the sample matrix, a positive control sample (PCS) can be prepared by spiking the solution with previously determined mycoplasma culture or standards. Do not use *Myco* Positive Control components in the detection kit for preparation. Follow the same pre-treatment process to make a RC-spiked positive

control solution;

(2) The positive control sample preparation should be processed carefully to prevent from the cross-contamination.

Digestion

Protocol A:

(1) Add 30 µL of Proteinase K Buffer, 50 µL of 5M NaCl and 20 µL of Proteinase K consecutively to each tube;

(2) Vortex the tubes for 5-10 seconds to mix the contents thoroughly;

Attention：

Do not mix the above three reagents together in advance. The appearance of flocs right after the addition of these reagents is normal, and will disappear after vortex and heating.

(3) Incubate the tubes at 70℃ for 15 minutes;

(4) Keep the tubes at room temperature for 5 minutes;

(5) Spin to collect the solution for 1-2 seconds;

Protocol B: Samples containing high concentration of protein, such as 5% HSA

(1) Add 30 µL of Proteinase K and 200 µL *Myco* Lysis Buffer 2 consecutively to each tube;

(2) Vortex the tubes for 5-10 seconds to mix the contents thoroughly;

(3) Incubate the tubes at 70℃ for 15 minutes;

(4) Keep the tubes at room temperature for 5 minutes;

(5) Spin to collect the solution for 1-2 seconds;

Note: Subsequent steps can be categorized into manual extraction and extraction using a nucleic acid extraction and purification instrument. After the step of digestion, the following nucleic acid extraction should be carried out promptly!

Manual Extraction Operation Procedure

Lysis & Binding

(1) Add 200 µL of *Myco* Lysis Buffer 2 and 300 µL of isopropanol to each tube;

(2) Vortex the tubes for 5-10 seconds to mix the solution thoroughly;

(3) Add 15 µL of *Myco* Magnetic Beads to each tube and vortex the tubes for 10 minutes;

Attention：

Before using Myco Magnetic Beads, a thorough vortex for 30 seconds is necessary. In case of multiple samples, to vortex the beads solution for each sample in prior to transfer helps

(4) Place the tubes on a magnetic rack for 2-5 minutes to let the beads assembled to form a tight pellet and the liquid clear;

(5) Carefully remove the liquid without touching the beads;

Wash w/ *Myco* Wash Buffer

(1) Add 700 µL of *Myco* Wash Buffer (check if ethanol has been added) to each tube;

(2) Vortex the tubes for 30 seconds to re-suspend the beads;

(3) After assemble the beads to the bottom by short-time spin，place the tubes on the magnetic rack for 2-5 minutes to clear the solution;

(4) Carefully remove the liquid without touching the beads;

Wash w/ 70% Ethanol

(1) Add 700 µL of 70% ethanol to each tube；

(2) Vortex for 30 seconds to re-suspend the beads;

(3) After assemble the beads to the bottom by short-time spin，place the tubes on the magnetic rack for 2-5 minutes to clear the solution;

(4) Keep the tubes on the magnetic rack and carefully remove the liquid without touching the beads.

(5) Repeat steps 1-5 to wash again;

(6) Keep the tube open for 5-10 minutes at room temperature to let the beads dry;

Note：Attention should be paid to the drying process. It is better to stop drying when the beads surface appears slightly moist. Over-dry may decrease the target recovery.

Elution

(1) Move the tubes to a regular rack and add 100 µL of *Myco* Elution Buffer to the beads;

(2) Vortex the tubes to re-suspend the beads;

(3) Incubate the tubes at 70℃ for 15 minutes after a short-time spin. Vortex the tubes every 5 minutes during the incubation;

(4) After incubation, let the tubes cool down to room temperature, spin the tubes to assemble the beads to the bottom;

(5) Keep the tubes on the magnetic rack for 2-5 minutes to separate the beads from the solution;

(6) Carefully transfer the liquid without touching the beads to a new tube as read-to-use test sample.

(7) Save the test sample at -80℃ to -20℃ in case that the assay is not performed right away.

Operation Notes

- During magnetic immobilization, you may rotate the tube slightly to help quick assembling of the beads. The immobilization time can also be appropriately adjusted according to the actual performance;
- Change the tips to avoid cross-contamination. Do not keep the lids open of each tubes at the same time to avoid cross contamination;
- After vortex, spin in a short time to collect liquid and beads to the bottom to decrease the loss of target DNA;
- Perform the DNA detection immediately after the sample DNA isolation.

Nucleic Acid Extraction and Purification Instrument Operation Procedure

Reagent dispensing (suitable for inline extractors) (adjust according to actual machine models, example models 1/5/7/11 are for heating columns)

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

(1) Column 1/7: Add 300 µL isopropanol, 15 µL *Myco* Magnetic Beads, and 200 µL *Myco* Lysis Buffer 2 to each well using protein digestion protocol A; add 300 µL isopropanol and 15 µL *Myco* Magnetic Beads to each well using protein digestion protocol B;

(2) Column 2/8: 700 µL *Myco* Wash Buffer per well (check for anhydrous ethanol addition before use);

(3) Column 3/9: 1 mL 70% ethanol per well;

(4) Column 4/10: 1 mL 70% ethanol per well;

(5) Column 5/11: 100 µL *Myco* Elution Buffer per well (Note: Add the liquid to the bottom of the deep well plate);

(6) Column 1/7: Add all samples after protein digestion (Note: Ensure there is no liquid residue).

Reagent Dispensing (applicable to rotary extraction instruments)

Pre-add the corresponding solutions according to the following deep-well plate arrangement, which includes:

(1) First plate (Banding): Add 300 µL of isopropanol, 15 µL of *Myco* Magnetic Beads, 200 µL of *Myco* Lysis Buffer 2, and all samples after protein digestion per well using protein digestion protocol A; add 300 µL of isopropanol, 15 µL of *Myco* Magnetic Beads, and all samples after protein digestion per well using protein digestion protocol B;

(2) Second plate (Wash1): 700 µL of *Myco* Wash Buffer per well (check for anhydrous ethanol addition before use);

(3) Third plate (Wash2): 1 mL of 70% ethanol per well;

(4) Fourth plate (Wash3): 1 mL of 70% ethanol per well;

(5) Fifth plate (Elution): 100 µL of *Myco* Elution Buffer per well (note: add the liquid to the bottom of the deep-well plate).

Program settings

Carry out the automated extraction experiment according to the following procedure;

Step	Well/Plate	Name	Waiting time (min:ss)	Mixing time (min:ss)	Magnetic Attraction Time (min:ss)	Mixing speed	Volume (μL)
1	1	Banding	00:00	10:00	00:50×3	Middle	1000
2	2	Wash1	00:00	01:00	00:50×3	Middle	700
3	3	Wash2	00:00	01:00	00:50×3	Middle	1000
4	4	Wash3	00:00	01:00	00:50×3	Middle	1000
5	5	Elution	03:00	10:00	00:50×3	Customize *	100
6	4	Discard	00:00	00:10	00:00	High	1000

* Mixed method: Bottom Mix 01:00 + Bottom Pause 09:00

Heating temperature setting: Elution temperature 70℃, start heating step for elution (Step 5);

When the automated program ends, transfer the elution from columns 5 and 11/plate 5 to clean nuclease-free centrifuge tubes for later use.

Operation Notes

- Shake and mix *Myco* Magnetic Beads before use, add the sample below the liquid surface to avoid the beads sticking to the deep-well plate walls;
- Add 100 μL *Myco* Elution Buffer to the bottom of the deep-well plate;
- It is recommended to aliquot the reagents first and then add the protein-digested samples at last;
- Negative and positive samples should be distributed in different columns or plates when plating; or nucleic acid extraction can be performed at different times;
- After adding the reagents, extraction should be performed immediately. If it needs to be hold, seal the plate with sealing film to prevent evaporation of ethanol and isopropanol;
- After extraction, immediately transfer the elution solution from the 5th and 11th columns/plate 5 to a clean nuclease-free centrifuge tube to avoid evaporation;
- Try to perform DNA detection immediately after completing nucleic acid extraction to ensure the accuracy of the test results.

DISCLAIMER

1. The product should be used according to the instructions in the manual. If the experimenter fails to operate according to the instructions, our company will not be responsible for any deviation in product performance caused by this.

2. The product is only used for scientific research and commercial production, and is not suitable for clinical diagnosis and treatment. Otherwise, all consequences arising shall be borne by the experimenter, and our company shall not be responsible.