Myco-spin™ Mycoplasma Extraction Kit

For the effective extraction Mycoplasma genomic DNA

RUO *Research Use Only* REF 17541 Σ 50 RT

PRODUCT FEATURES

- **Myco-spin™ Mycoplasma Extraction Kit** is designed for the effective extraction of DNA from mycoplasma infected cell.
- Effective isolate genomic DNA from samples purely ensure accuracy and repeatability of analysis more than other isolate methods in Mycoplasma detection test.
- The fast, simple procedure for extracting the high quality genomic DNA.

INTRODUCTION

Infected cell-line by mycoplasma may cause critical problems for cell-based research ,such as cell therapy and biopharmaceutical production. Up to 30 ~ 85% of cell cultures may be contaminated with mycoplasmas, the main contaminants being the species *M. orale, A. laidlawii, M. arginini, M. pneumoniae, M. fermentans, M. hyorhinis.* Testing for mycoplasma by efficient mycoplasma nucleic acid extraction is an essential quality control tool to assure accurate research and reliable biotechnological products.
The Myco-spin™ Mycoplasma Extraction Kit is fast and easy methods for extracting mycoplasma infected cell. The kit is suitable product for the nucleic acid extraction of mycoplasma species that are most commonly encountered in cell culture.

APPLICATION

Mycoplasma genomic DNA extracted using the Myco-spin™ Mycoplasma Extraction Kit is suitable for use in a cell-based research :

- ✓ For research Use Only, Not for use in diagnostic procedures.
- ✓ The Kit is developed, designed, and sold for research purpose only.
- ✓ In-process monitoring for the presence of Mycoplasma

KIT CONTENTS

Components	50 prep
Buffer ML1	25 ml
Buffer ML2	25 ml
Buffer MWA	40 ml
Buffer MWB	10 ml / 5 ml
Buffer ME	20 ml
Spin Column & Collection Tube	50 ea
RNase A, lyophilized[1]	3 mg x 1 vial
Proteinase K, lyophilized[1]	22 mg x 1 vial

[1] The lyophilized RNase A and Proteinase K be stored at RT (15 – 25℃).
Dissolved (in D.W) Enzyme should be immediately stored at −20℃.

STORAGE CONDITION

The Myco-spin™ Mycoplasma Extraction Kit can be stored at room temperature (15-25℃). Under these conditions, the kit can be stored for up to 24 months without any reduction in performance and quality. The lyophilized RNase A and Proteinase K can be stored at room temperature. Dissolved RNase A and Proteinase K should be immediately stored at −20℃. These solutions are stable at −20℃ for up to 24 months and 20 times frozen-thawing until the kit expiration date.

IMPORTANT NOTES

1. Check Buffer ML1 or Buffer ML2 before use for salt precipitation. Dissolve by heating to 70℃ with gentle agitation.
2. All centrifugation steps are carried out at 13,000 rpm (~13.400 x g) in table-top micro-centrifuge at room temperature (15 - 25℃).
3. Buffer MWB is supplied as concentrate. Before using for the first time, be sure to add absolute ethanol (96 - 100 %) to obtain a working solution.

ADDITIONAL REQUIREMENTS

1. Ethanol (EtOH) > 96 % abs.
2. Micro-centrifuge and heat block for 1.5 (2.0) ml reaction tubes
3. Vortex mixer
4. Other general lab equipments

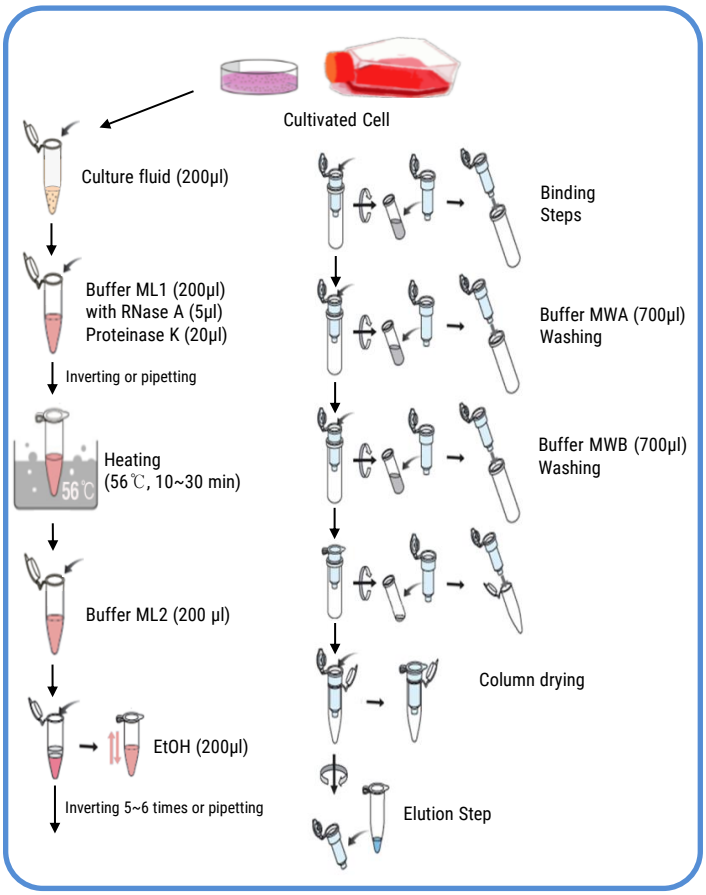
PRODUCT WARRANTY AND SATISFACTION GUARANTEE

At iNtRON we pride ourselves on the quality and availability of our technical support. Our CRT center is staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology in the use of iNtRON products. If a iNtRON product does not meet your expectations, simply call your local distributor. If you have questions about product specifications or performance, please call iNtRON Technical Services or your local distributor.

NOTICE BEFORE USE

Myco-spin™ Mycoplasma Extraction Kit is intended for Research Use Only. This product is not intended for the diagnosis, prevention, or treatment of disease. All care and attention should be exercised in the handling of the products. Do not use internally or externally in humans or animals. Please observe general laboratory precaution and utilize safety while using this kit.

BRIEF PROCEDURE



PROTOCOL

Recommendation before use.

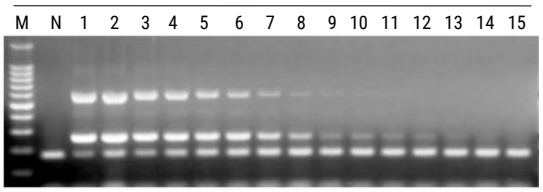
- PCR inhibiting substances may accumulate over time in cell culture medium.
- Medium with more than 10~12% serum has inhibitory effects on downstream application such as PCR. Moreover, phenol red, a routine material in cell culture medium, is likely to cross-react and thus interfering signals in PCR.
- These negative effects can be overcame by using the Myco-spin™ Mycoplasma Extraction Kit for sample preparation.

1. Transfer the approximately 200µl of culture fluid (0.5 ~ 1 x 10^5 cells) to a new 1.5 ml micro-centrifuge.
2. Add 200 µl Buffer ML1, 20 µl Proteinase K and 5 µl RNase A Solution into sample tube and mix by inverting or pipetting.
3. Incubate the lysate at 56℃ (pre-heated heat block or water bath) for 10 – 30 min.
4. After lysis completely, add 200 µl of Buffer ML2 into upper sample tube and mix thoroughly.
5. Add 200 µl of absolute ethanol into the lysate, and mix well by gently inverting 5 – 6 times or by pipetting. DO NOT vortex. After mixing, briefly centrifuge the 1.5 ml tube to remove drops from inside of the lid.
6. Carefully apply the mixture from step 5 to the Spin Column (in a 2 ml Collection Tube) without wetting the rim, close the cap, and centrifuge at 13,000 rpm for 1 min. Discard the filtrate and place the Spin Column in a 2 ml Collection Tube (reuse).
7. Add 700 µl of Buffer MWA to column and centrifuge for 1 min at 13,000 rpm.
8. Add 700 µl of Buffer MWB to the Column without wetting the rim, and centrifuge for 1 min at 13,000 rpm. Discard the flow-through and place the Column into a 2.0 ml Collection Tube (reuse), Then again centrifuge for additionally 1 min to dry the membrane. Discard the flow-through and Collection Tube altogether.
9. Place the Spin Column into a new 1.5 ml tube (not supplied), and 50 µl of Buffer ME directly onto the membrane. Incubate for 1 min at room temperature and then centrifuge for 1 min at 13,000 rpm to elute. **Note** : (Optional) Preheat Buffer ME to 50 - 70℃ for elute.

TECHNICAL INFORMATION

❖ Application of Mycoplasma gDNA extracted with Myco-spin™ Mycoplasma Extraction Kit.

Detection test of *M.fermentans*-infected K562 cells



(Amount of genomic DNA used for each lane)

Lane	M		N	1	2	3	4	5	6
gDNA	DNA marker		0 ng	100 ng	50 ng	25 ng	12.5 ng	6.3 ng	3.2 ng
Lane	7	8	9	10	11	12	13	14	15
gDNA	1.6 ng	800 pg	400 pg	200 pg	100 pg	50 pg	25 pg	12.5 pg	6.25 pg

Fig.1. Result of determining minimal required amount of genomic DNA per test using e-Myco™ Plus Mycoplasma PCR Detection Kit (Cat.NO. 25237).
Cultured cell were extracted with Myco-spin™ Mycoplasma Extraction Kit, and the sample were used ½ diluted with PBS buffer. After, extracted genomic DNA were used as template of PCR analysis.

❖ Supplementary Data of the Kit's applications

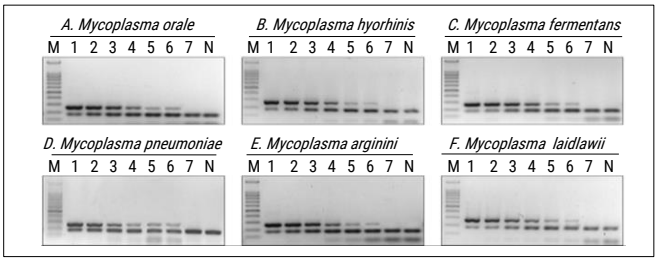


Fig.2. Application of e-Myco™ VALiD Mycoplasma PCR Detection Kit (Cat.NO. 25237).
Amplification of a 10-fold serial dilution of Mycoplasma gDNA template. (1×10^6 ~ 1 cfu/ml)

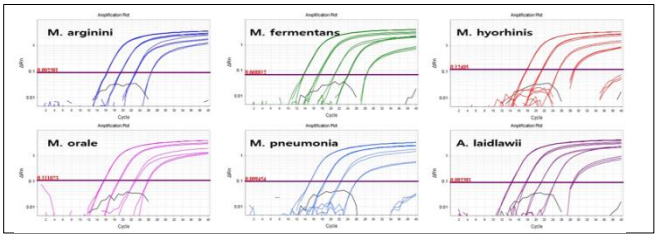


Fig.3. Application of e-Myco™ VALiD-Q Mycoplasma PCR Detection Kit (Cat.NO. 25245).
Amplification of a 10-fold serial dilution of Mycoplasma gDNA template. (1×10^5 ~ 10 cfu/ml)

TROUBLE SHOOTING GUIDE

Problem	Possible Cause	Recommendation
Low DNA yield	Low concentration of cells in the sample	• Concentrate a larger volume of a new cell-free sample to 200 µl using a Centricon®-100 (Amicon, USA). Repeat the DNA purification procedure by adding 5–10 µg of carrier to each lysate if the sample has a low DNA content.
	Inefficient cell lysis due to insufficient mixing with Buffer ML2	• Repeat the DNA purification procedure with a new sample. Be sure to mix the sample and Buffer ML2 immediately and thoroughly by pulse-vortexing.
	Low-percentage ethanol used instead of 100%.	• Repeat the purification procedure with a new sample. Do not use denatured alcohol, which contains other substances such as methanol or methyl-ethyl-ketone
	pH of water incorrect (acidic)	• Low pH may reduce DNA yield. Ensure that the pH of the water is at least 7.0 or use Buffer ME for elution.
White precipitate in Buffer ML1 or Buffer ML2	White precipitate may be formed after storing at low temperature or prolonged storage	• Any precipitate in Buffer ML1 or Buffer ML2 must be dissolved by incubation of the buffers at 56°C. The precipitate has no effect on kit's function. Dissolving the precipitate at high temperature will not compromise yield or quality of the purified nucleic acid.
General handling	Lysate has not completely passed through the membrane	• Using spin protocol: Centrifuge for 1 min at full speed or until all the lysate has passed through the membrane.

ORDERING INFORMATION

Product Name	Amount	Cat. No.
e-Myco™ plus Mycoplasma PCR Detection Kit	48 Tubes	25237
e-Myco™ VALiD Mycoplasma PCR Detection Kit	48 Tests	25239
e-Myco™ Mycoplasma PCR Detection Kit (ver.2.0)	48 Tubes	25235
e-Myco™ VALiD-Q Mycoplasma qPCR Detection Kit	50 Tests	25245
MycoClean™ Mycoplasma Prevention Spray	200 ml	21083
M-Solution™ 1-2 Antibiotic for Mycoplasma	10 ml (each)	21081

Technical support : +82-505-550-5600

Review date : 2018. 04. 25