MagicPure® Mycoplasma DNA Kit

Please read the datasheet carefully prior to use.

Cat. No. EH401

Version No. Version 1.1

Storage: at room temperature (15°C-25°C) for one year. Carrier RNA (1 μg/μl) should be stored at -20°C, avoid repeated freezing and thawing.

Description

This kit utilizes a unique lysis buffer to lyse mycoplasma cells from ≤400 μl biological samples, and specifically adsorb and purify mycoplasma DNA based on silicon-based magnetic beads. Purified DNA with high purity is suitable for mycoplasma detection experiment with *TransDetect*® qPCR Mycoplasma Detection Kit (FM321). This kit is suitable for high-throughput automated nucleic acid extractor adopting magnetic rod technology.

Features

- Simple and fast, no centrifugation required.
- High yield and high purity.

Kit Contents

Component	EH401-01/11 (50 rxns)
Binding Buffer 50 (BB50)	11 ml
Clean Buffer 50 (CB50)	25 ml
Wash Buffer 50 (WB50)	12 ml
Elution Buffer (EB)	10 ml
Proteinase K (20 mg/ml)	1 ml
Magnetic Mycoplasma Beads	1 ml
Carrier RNA (1 μg/μl)	100 μl
Magnetic Stand (16 hole)	1/-

Reagents and nstruments that are not included in the kit:

1 M HCl, 1 M NaOH, isopropanol (analytically use), high-speed centrifuge, thermostatic metal bath, vortex oscillator

Sample Requirements

- Sample should avoid repeated freeze-thaw.
- Samples containing cells: the number of cells should not be higher than 1×10^6. Cells should be enriched using a centrifuge prior to extraction, followed by 400 μl of supernatant for extraction.
- Cell-free samples: 400 μl of sample for extraction.
- If the sample volume is higher than 400 μl, concentrate to 400 μl before extraction.
- When the sample is a biological intermediate, please ensure that the pH value is neutral. Use sodium hydroxide or hydrochloric acid to adjust the pH of the sample to neutral (pH 6.0-8.0) before extraction.
- To ensure reliable results:

  It is recommended to add negative control samples (NCS), and simultaneously perform nucleic acid extraction and quantitative detection steps. It is used to determine the presence or absence of process contamination in nucleic acid extraction.

  It is recommended that all samples to be extracted be added to internal control (IC). It is used to determine whether there are inhibitor residues in nucleic acid extraction.

Procedures

Add different volumes of absolute ethanol to CB50 and WB50 before use.

Component	EH401-01/11
Clean Buffer 50 (CB50)	25 ml
Wash Buffer 50 (WB50)	48 ml

All magnetic separation is performed at room temperature, and the magnetic beads are mixed by vortexing before use.

Negative control sample (NCS): take 400 µl of 1×TE buffer or DNA dilution buffer as a single NCS.

Internal control (IC) working solution: pipet the IC included in the mycoplasma detection kit (FM321)and Carrier RNA into a 1.5 ml centrifuge tube according to the ratio in the table below , and mix well for later use.

Number of extractions	IC	Carrier RNA
10 rxns	100 µl	20 µl
50 rxns	500 µl	100 µl

1. Single sample extraction: add 12 µl IC working solution, 20 µl Proteinase K, and 400 µl of sample to be tested into a 1.5 ml centrifuge tube.
   Single NCS extraction: add 12 µl IC working solution, 20 µl Proteinase K, and 400 µl negative control sample(NCS) into 1.5 ml centrifuge tube.
2. Add 200 µl of lysate BB50 and mix well by vortexing.
3. Incubate at room temperature for 10 minutes, during which mix well by vortexing 1-2 times.
4. Add 200 µl of isopropanol, mix well by vortexing for 10 seconds, and add 20 µl of magnetic bead suspension (Note: mix well by vortexing before using the magnetic beads).
5: Mix well by vortexing for 1 minute and keep it still for 2 minutes.
6. Repeat step 5 three times.
7. Place the centrifuge tube on the magnetic stand to perform magnetic separation, pipet the liquid other than the magnetic beads, and avoid pipetting the magnetic beads. (Suggestions for magnetic separation operation: Spin in a handheld centrifuge for 10 seconds to bring down the liquid on the tube cover to the bottom of the tube, and keep it still on a magnetic stand for 30 seconds.)
8. Take the centrifuge tube down, add 800 µl CB50 (check if absolute ethanol has been added before use), vortex and mix for 2 minutes, then perform magnetic separation. Pipet the liquid other than the magnetic beads, and avoid pipetting the magnetic beads. (Suggestions for magnetic separation operation: Spin in a handheld centrifuge for 10 seconds to bring down the liquid on the tube cover to the bottom of the tube, and keep it still on a magnetic stand for 30 seconds.)
9. Take the centrifuge tube down, add 500 µl WB50 (check if absolute ethanol has been added before use), vortex and mix for 2 minutes, then perform magnetic separation. Pipet the liquid other than the magnetic beads, and avoid pipetting the magnetic beads. (Suggestions for magnetic separation operation: Spin in a handheld centrifuge for 10 seconds to bring down the liquid on the tube cover to the bottom of the tube, and keep it still on a magnetic stand for 30 seconds.)
10. Repeat step 9 once. (Suggestions for discarding: discard all the liquid at the bottom of the tube again with a small volume pipette. Liquid residue will affect downstream testing results.)
11. Place the centrifuge tube on the magnetic stand and let dry at room temperature for 8-10 minutes.
12. Take the centrifuge tube down, add 100 µl EB, thoroughly mix by pipetting, incubate at 65°C for 5-10 minutes, and mixing by pipetting twice during the incubation period.
13. Place the centrifuge tube on the magnetic stand for magnetic separation, and pipet the liquid other than the magnetic beads into a sterile 1.5 ml centrifuge tube, avoid pipetting the magnetic beads. Store DNA at -20°C.

Notes

- Avoid repeated thawing and freezing samples to ensure high-quality extracted nucleic acid.
- Use Nuclease-free sterile microcentrifuge tubes and pipette tips to avoid DNase contamination.
- Be sure to vortex and mix the beads before use.
- To avoid contamination between samples, please disinfect the environment and replace the pipette tip immediately.

For research use only, not for clinical diagnosis.

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