Mycoplasma DNA Sample Preparation Kit (Magnetic Beads)

Catalog Number：OPA-E101

Assay Tests: 50 Preps

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures

IMPORTANT: Please carefully read this user guide before performing your experiment.

Product Information

The Mycoplasma DNA Sample Preparation Kit (Magnetic Beads) is designed for extraction of Mycoplasma DNA from biopharmaceutical products.

Use the kit to extract mycoplasma DNA before you detect mycoplasma DNA from test samples. For the detection kit information, please refer to the user guide of Mycoplasma Rapid Detection Kit (OPA-S101) (ACROBiosyestems.com).

This kit isolates mycoplasma DNA from biopharmaceutical samples using magnetic beads. The process typically involves lysing the sample to release the DNA, then using magnetic beads coated with a DNA-binding agent to selectively bind the DNA. The beads are then separated from the mixture using a magnetic stand, and the DNA can be washed and eluted off the beads for further analysis or use. This kit applies to extract trace mycoplasma DNA from complex matrix samples including cell banks, virus seed lots, cell and gene therapy products, raw materials, ancillary materials and other biopharmaceutical products. This kit helps to get high purity and stable quality mycoplasma DNA, which can be used for mycoplasma qPCR detection. It is recommended to be used in combination with OPA-S101 Mycoplasma Rapid Detection Kit (qPCR) for detection of mycoplasma contamination in biopharmaceutical products.

Contents and Storage

The kit can be used for 50 preps of mycoplasma DNA extraction from test samples.

Contents	Amount	Storage
Buffer AL	35 mL	10℃ to 30℃ **Note**: The Proteinase K and MagBeads Suspension can be stored in ambient temperature (10 to 30℃). For optimal long-term stability, these two components are recommended to be stored in 2-8℃
Proteinase K	4 mL	
MagBeads Suspension (MB)	1.4 mL	
CR Powder	310 μg	
Buffer WA	38 mL	
Buffer WB	18 mL	
Buffer MEB	4 mL	
Sample Dilution Buffer	5 mL	

The unopened kit is stable for 12 months from the date of manufacture if stored at 10°C to 30°C.

Required materials not supplied.

Equipments	Magnetic stand
	Block heater
	Mini centrifuge
	Vortex
	Pipettors: P1000, P200, P100, P10
Reagents	Ethanol, 99.7%
	DNase/RNase-free ddH_2O
Consumables	Disposable gloves
	Nuclease-free, DNA-free aerosol-resistant pipet tips
	Low DNA-Binding Microcentrifuge Tubes (Nuclease-free, DNA-free)

Workflow

Prepare reagents and samples

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Preparation of Negative Extraction Control (NEC) or Positive Extraction Control (PEC) (IMPORTANT)

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Digest the test samples and controls

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Bind and wash the DNA

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Elute the DNA

Prepare the reagents and samples.

Prepare the reagents: before first use of the kit.

1. Incubate the MagBeads Suspension at room temperature for 30 minutes, or until the beads are completely suspended.
2. Refer to the bottle label, add amount of 99.7% ethanol to bottle of Buffer WA or WB, then mix completely.
3. Label the bottle to indicate that it contains ethanol, then store the bottle at room temperature.
4. Preparation of CR Solution: Briefly centrifuge the CR Powder tube, then add 310 µL DNase/RNase-free ddH_2O to the tube, and vortex thoroughly.

   ***NOTE**:*

   The CR Solution should be stored at -20℃, it can be divided into small portions to avoid freeze-thaw cycles.

Prepare the samples.

The sample volume for extraction is 200 µL, each sample for extraction (including all samples to be tested, the negative extraction control and the positive extraction control) should be added with **7 µL Internal Control DNA** (IC, provided in OPA-S101). Prepare the different types of samples according to following instructions:

1. Insufficient volume samples: If sample volume is less than 200 µL (total cell number $\leqslant 10^7$), add some sample dilution buffer or 0.9% sterile NaCl solution (customer-self prepared) to make the volume reach to 200 µL.
2. Cell suspension: If the total cell number is no more than 10^7, mix the sample thoroughly and take 200 µL for extraction. If the total cell number is greater than 10^7, centrifuge the cell suspension at 100×*g* for 5 minutes, transfer the supernatant to a new microfuge tube without disturbing the sediment. If the volume of supernatant is greater than 200 µL, centrifuge it at 20000×*g* for 10 minutes, then remove supernatant until the remaining volume is approximately 200 µL, then mix it thoroughly.
3. Cell supernatant or used cell culture medium: When the volume of cell supernatant

or used cell culture medium is greater than 200 µL, centrifuge it at 20000×*g* for 10 minutes, then remove supernatant until the remaining volume is approximately 200 µL, then mix it thoroughly.

4. Non-cellular sample: If sample is in the state of powder, please dissolve the sample with sample dilution buffer and dilute it to 1 mg/mL~100 mg/mL, then take 200 µL of sample for extraction.
5. Preparation of Negative Extraction Control (NEC): Label low DNA-binding 2.0 mL microfuge tubes "NEC", add 200 µL of fresh matrix solution (the same buffer with samples to be tested) or sample dilution buffer provided in this kit.
6. Preparation of Positive Extraction Control (PEC): Label low DNA-binding 2.0 mL microfuge tubes "PEC", add **70 µL** Positive Control DNA (PC, provided in OPA-S101: Mycoplasma Rapid Detection Kit(qPCR)), **130 µL** of fresh matrix solution (the same buffer with samples to be tested) or sample dilution buffer provided in this kit.

Digest the test samples and controls.

1. Label low DNA-binding 1.5/2.0 mL microfuge tubes “Sample”, “NEC”, “PEC”.
2. Add 207 µL (including the volume of sample and IC) of samples or controls to each tube.
3. Add 70 µL of Proteinase K to each tube, vortex for 30 seconds and briefly centrifuge.
4. Incubate the tubes at 60°C for 8 minutes on a block heater, with vortexing at 1000 rpm. If available, set heater lid to 70°C.
5. Briefly centrifuge, and cool samples to room temperature.

Bind the DNA

1. Add 600 µL of Buffer AL to each tube, then close the cap and invert five times to mix, vortex for 1 minute and briefly centrifuge.
2. Add 25 µL of MagBeads Suspension and 3 µL of CR Solution to each tube, then close the cap and invert five times to mix.

   NOTE: The MagBeads Suspension should be resuspended before use.
3. Vortex all the tubes for 1 minute.
4. Let the tubes stand for 10 minutes, and then vortex for 20 seconds in every 3 minutes.
5. Briefly centrifuge and place the tubes in the magnetic stand with the pellet against the magnet, then let the tubes stand for 3 minutes or until the solution is clear.
6. Without disturbing the magnetic beads, remove the supernatant using a pipette or by aspiration.

Wash the DNA

1. Add 800 µL Buffer WA to each tube, then vortex for 10 seconds.
2. Briefly centrifuge the tubes, then place the tubes in the magnetic stand, let the tubes stand for 2 minutes or until the solution is clear.
3. Without disturbing the magnetic beads, remove the supernatant using a pipette or by aspiration.
4. Add 800 µL Buffer WB to each tube, then vortex for 10 seconds.

5. Briefly centrifuge the tubes, then place the tubes in the magnetic stand, let the tubes stand for 1 minute or until the solution is clear.
6. Without disturbing the magnetic beads, remove the supernatant using a pipette or by aspiration.
7. Repeat the steps 4-6.
8. Briefly centrifuge the tubes, then place the tubes in the magnetic stand, let the tubes stand for 30 seconds or until the solution is clear.
9. Use a P10 to remove the remaining solution from the bottom of the tube.
10. With the tube lid open, air-dry the Magnetic beads in the magnetic stand for no more than 2-3 minutes at room temperature.

Note: Do not over-dry; the bonded DNA are not easily eluted from the over-dried beads.

Elute the DNA

1. Add 70 µL of Buffer MEB to each tube, then resuspend the beads by vortexing for 15-20 seconds or pipetting up and down until suspension is fully homogenized.
2. Incubate the tubes at 80°C for 10 minutes on a block heater, with vortexing at 1000 rpm.
3. Briefly centrifuge the tubes for 15 seconds, then place the tubes in the magnetic stand, let the tubes stand for 1-3 minutes or until the solution is clear.
4. Use a P100 to transfer the liquid phase to a new 1.5 mL microcentrifuge tube.

Note: Do not disturb the magnetic beads.

The purified, high-quality eluted DNA is ready to use in demanding downstream applications.

Store eluted DNA for up to 24 hours at 2°C to 8°C or for long time at −20°C.