# 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.

# Contents

## Product Information

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

Before detecting the mycoplasma DNA, use the Mycoplasma DNA Sample Preparation Kit to extract nucleic acids from test samples. For detection information, please refer to the user guide of Mycoplasma Rapid Detection Kit (OPA-S102) (ACROBiosystems.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 bank, virus seed bank, 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. This Mycoplasma DNA Sample Preparation Kit is suitable for use in combination with Mycoplasma Rapid Detection Kit(qPCR) (OPA-S102) for qualitative detection of mycoplasma contamination in biopharmaceutical products.

This instruction included the process of manual DNA extraction and automated DNA extraction. About automatic DNA extraction, we provide a program for KingFisher™ Flex.



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## 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℃<br>**Note**:<br>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 at 2℃ to 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 reagents can be stored stably for 12 months from the date of manufacture when kept at a storage temperature between 10℃ and 30℃.

In an environment with a low temperature ( < 20℃), precipitates may form in Buffer AL and Buffer WA. Place the reagent bottles in a water bath at 37℃-50℃ for about 10 minutes until the precipitates are completely dissolved before use.



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# PART A: Manual DNA extraction

## Required materials not supplied

| | |
|---|---|
| **Equipment** | Magnetic stand |
| | Block heater |
| | Mini centrifuge |
| | Vortex |
| | Pipettors: P1000, P200, P100, P10 |
| **Reagents** | Ethanol, 99.7% |
| | DNase/RNase-free dd$H_2O$ |
| **Consumables** | Disposable gloves |
| | Nuclease-free, DNA-free aerosol-resistant pipet tips |
| | Low DNA-Binding Microcentrifuge Tubes (Nuclease-free, DNA-free) |



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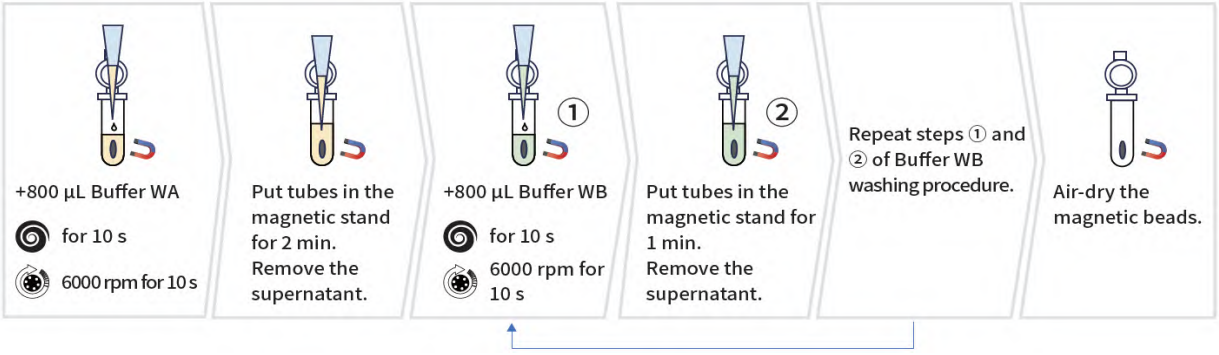
## Workflow for manual DNA extraction

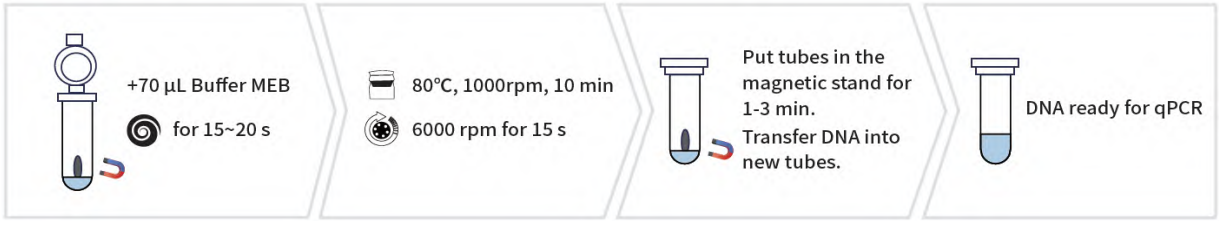
### Step 1 Digest samples


### Step 2 Bind the DNA


### Step 3 Wash the DNA




### Step 4 Elute the DNA




+ add  vortex  centrifuge  incubate



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## Prepare the reagents and samples for manual DNA extraction

### 1. Prepare the reagents: before first use of the kit.

1.1 Incubate the MagBeads Suspension at room temperature for 30 minutes in advance. Before being used, the magnetic beads have to be vigorous vortexed and mixed.

**NOTE**: The MagBeads Suspension should be mixed for at least 1 minute until they are evenly dispersed.

Invert the tube several times and observe to confirm the state of the magnetic beads: There should be no magnetic beads adhering to the bottom of the tube, and the magnetic bead suspension should be in a uniformly dispersed state with a consistent color and no dark-colored aggregated lumps.

1.2 Refer to the bottle label, add 40 mL of 99.7% ethanol to the bottle of Buffer WA, then mix completely.

1.3 Refer to the bottle label, add 80 mL of 99.7% ethanol to bottle of Buffer WB, then mix completely.

1.4 Label the bottle to indicate that it contains ethanol, then store the bottle at room temperature.

1.5 Preparation of CR Solution: Briefly centrifuge the CR Powder tube, then add 310 µL DNase/RNase-free dd$H_2O$ to the tube, and vortex thoroughly.

**NOTE**: The CR Solution should be stored at -20℃, and aliquot the CR solution into small volumes to avoid more than three freeze-thaw cycles.

### 2. Prepare the samples.

The volume for each extraction is 207 µL, which includes 200 µL of sample and **7 µL Internal Control DNA** (IC, provided in kit OPA-S102). To ensure the accuracy results of mycoplasma detection, different types of samples are recommended to be pre-treated according to different instructions as followings:



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2.1 **Small-volume samples:** When the volume of the sample to be tested is less than 200 µL (cell concentration ≤ $10^6$ cells/mL), the sample can be made up to 200 µL with Sample Dilution Buffer or 0.9% sterile NaCl solution (provided by the customer). Take 200 µL of the sample or make up the volume to 200 µL for extraction.

2.2 **Cell suspension:**

2.2.1 When the cell concentration is no more than $10^6$ cells/mL, mix the sample thoroughly and take 200 µL of the sample for extraction.

2.2.2 When the cell concentration is greater than $10^6$ cells/mL, centrifuge the cell suspension at 100×*g* for 5 minutes, transfer the supernatant to a new microfuge tube without disturbing the pellet. When the volume of the supernatant is greater than 200 µL, centrifuge it at 20000×*g* for 10 minutes to enrich the mycoplasma. Remove the supernatant immediately after centrifugation until the remaining volume is approximately 200 µL, and mix the remaining sample thoroughly for extraction.

2.3 **Cell supernatant or used cell culture medium:** When the volume of the cell supernatant or used cell culture medium is greater than 200 µL, centrifuge it at 20000 ×*g* for 10 minutes to enrich the mycoplasma. Remove the supernatant immediately after centrifugation until the remaining volume is approximately 200 µL, and mix the remaining sample thoroughly for extraction.

2.4 **Non-cellular sample:** When the sample is in powder form, please dissolve the sample with the Sample Dilution Buffer and dilute it to 1 mg/mL~100 mg/mL, then take 200 µL of the sample for extraction.

2.5 **Preparation of Negative Extraction Control (NEC):** Label a low DNA-binding 2.0 mL microfuge tube as "NEC", and add 200 µL of fresh matrix solution (the same buffer as that of the samples to be tested) or sample dilution buffer provided in this kit.

2.6 **Preparation of Positive Extraction Control (PEC):** Label a low DNA-binding 2.0 mL microfuge tube as "PEC", add **70 µL** of Positive Control DNA (PC, provided in the kit



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OPA-S102), and then add **130 µL** of fresh matrix solution (the same buffer as that of the samples to be tested) or the Sample Dilution Buffer provided in this kit.

## Protocol for manual DNA extraction

### 1. Digest the test samples and controls.

1.1 Label low DNA-binding 1.5/2.0 mL microfuge tubes as “Sample”, “NEC” and “PEC”.

1.2 Add 207 µL (including the volume of sample and IC) of samples and controls to each tube.

1.3 Add **70 µL of Proteinase K** to each tube, vortex for 30 seconds and briefly centrifuge.

1.4 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.

1.5 Briefly centrifuge, and cool the samples to room temperature.

**NOTE:** Briefly centrifuge: It is recommended to centrifuge at 3000-6000rpm for 5-10 seconds. It is applicable to all of briefly centrifuge mentioned in this instruction manual.

### 2. Bind the DNA

2.1 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.

2.2 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**: (1) To ensure the accuracy of the magnetic beads volume added, please vortex the MagBeads Suspension for at least 1 minute before used.

(2) After adding 3-4 samples, please vortex the suspension again for about 30 seconds before continuing to add samples.

(3) To ensure uniformity, it is recommended that when aspirating the magnetic beads each time, you can gently pipette up and down twice with



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a pipette before aspiration.

2.3 Vortex all the tubes for 1 minute.

2.4 Let the tubes stand for 10 minutes, and then vortex for 20 seconds in every 3 minutes.

2.5 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.

2.6 Without disturbing the magnetic beads, remove the supernatant using a pipette or by aspiration.

## 3. Wash the DNA

3.1 Add **800 µL Buffer WA** to each tube, then vortex for 10 seconds.

3.2 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.

3.3 Without disturbing the magnetic beads, remove the supernatant using a pipette or by aspiration.

3.4 Add **800 µL Buffer WB** to each tube, then vortex for 10 seconds.

3.5 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.

3.6 Without disturbing the magnetic beads, remove the supernatant using a pipette or by aspiration.

3.7 Repeat the steps 3.4-3.6.

3.8 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.

3.9 Use a P10 to remove the remaining solution from the bottom of the tube.

3.10 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:** (1) Residual ethanol will interfere with the subsequent PCR reaction, please ensure that the ethanol is completely volatilized. Do not over-dry the



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magnetic beads, or the bonded DNA are not easily eluted.

(2) Observe the magnetic beads under bright light, add the Buffer MEB immediately when the surface of the magnetic beads appears dull.

(3) If the surface of the magnetic beads is yellowish-brown, it indicates excessive drying, which will affect the recovery rate of nucleic acid.

## 4. Elute the DNA

4.1 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.

4.2 Incubate the tubes at 80°C for 10 minutes on a block heater, with vortexing at 1000 rpm.

4.3 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.

4.4 Use a P100 to transfer the liquid phase to a new 1.5 mL microcentrifuge tube.

**NOTE:** Do not disturb the magnetic beads.

## 5. DNA Sample Storage

5.1 The purified, high-quality eluted DNA is ready to use in demanding downstream applications. It is recommended to finish qPCR testing within 24 hours, and the DNA can be temporarily stored at 2°C to 8°C. For long-term storage, it should be placed at −20°C, and you should complete the DNA testing within 7 days.



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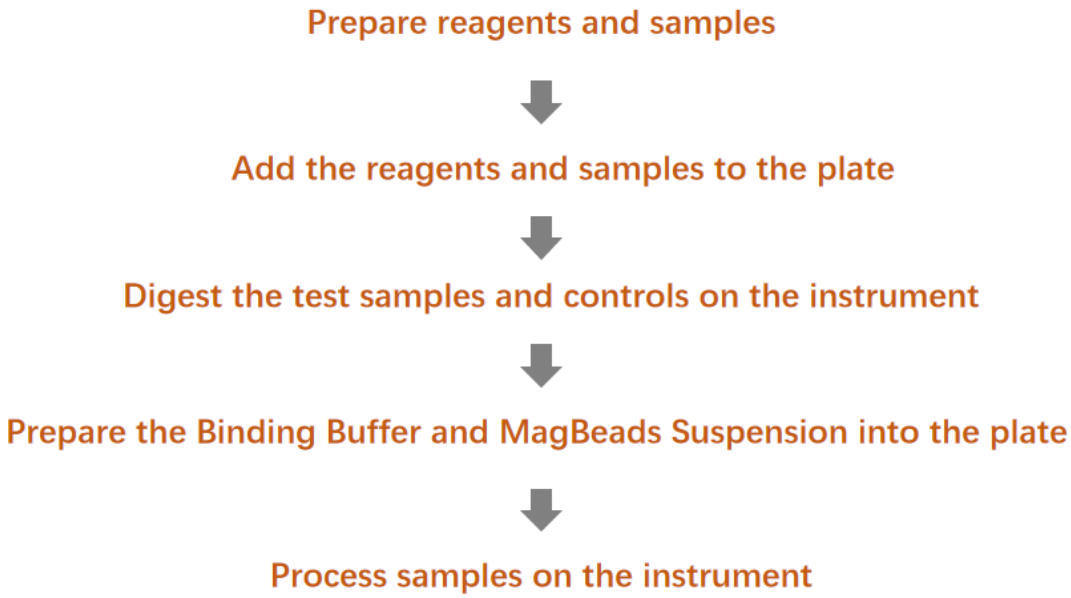
# PART B: Automated DNA extraction: Apply to KingFisher™ Flex

## Required materials not supplied for KingFisher™ Flex

| | |
|---|---|
| **Equipment** | Mini centrifuge |
| | Vortex |
| | Automated extraction instrument (KingFisher™ Flex) |
| | Pipettors: P1000, P200, P100, P10 |
| **Reagents** | Ethanol, 99.7% |
| | DNase/RNase-free dd$H_2O$ |
| **Consumables** | Disposable gloves |
| | Nuclease-free, DNA-free aerosol-resistant pipet tips |
| | Low DNA-Binding Microcentrifuge Tubes (Nuclease-free, DNA-free), 96 Deep-well plate, 96-Strip Tip Comb |

## Workflow for automated DNA extraction by KingFisher™ Flex



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## Prepare the reagents and samples for automated DNA extraction by KingFisher™ Flex

**1. Prepare the reagents: before first use of the kit.**

1.1 Please refer to manual DNA extraction, point 1 on page 5.

**2. Prepare the samples.**

2.1 Please refer to manual DNA extraction, point 2 on page 5.

## Protocol for automated DNA extraction by KingFisher™ Flex

The following steps apply for KingFisher™ Flex (96-well plate).

**1. Reagents dispensing**

1.1 Prepare 6 KingFisher™ Flex 96 deep-well plates, label the plates as: **96 tip comb plate**, **Lysis plate**, **Wash1 plate**, **Wash2 plate**, **Wash3 plate** and **Elute plate**.

1.2 Put a KingFisher™ Flex 96-strip tip comb into the 96 tip comb plate.

1.3 Add **207 µL** (including the volume of sample and IC) of samples or controls to the wells of Lysis plate, then add **70 µL of Proteinase K** to each well.

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**NOTE:** The Internal Control (IC) is provided in the Mycoplasma Rapid Detection Kit (qPCR) (Cat. No.: OPA-S102).

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1.4 Add **800 µL of Buffer WA** to each well in **Wash1 plate**.

1.5 Add **800 µL of Buffer WB** to each well in **Wash2 plate**.

1.6 Add **800 µL of Buffer WB** to each well in **Wash3 plate**.

1.7 Add **100 µL of Buffer MEB** to each well in **Elute plate**.

**2. Automated Extraction Process**

2.1 Clean the work space with 75% ethanol before use.

2.2 Open the software **Thermo Scientific BindIt**, and open the procedure **Acro-OPA-E101.bdz**. Click "**Start**".



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2.3 Put the plate in the specified sequence.

**NOTE:** Please check all the plates are placed correctly and the 96-strip tip comb are inserted before running.

2.4 Run the procedure.

2.5 Prepare the binding buffer according to the number of samples being extracted. Prepare a 50 mL tube for binding buffer using reagents and volumes shown in the table below.

**IMPORTANT!** To compensate for pipetting losses, it is recommended that the **N** is equals to the number of extracted samples plus 1 or 2.

| Kit Reagents | Volume for 1 sample | Volume for Binding Buffer |
|---|---|---|
| Buffer AL | 600 µL | 600 µL×**N** |
| CR Solution | 3 µL | 3 µL×**N** |
| Total | 603 µL | 603 µL×**N** |

2.6 After the sample digestion is finished, the procedure will be paused automatically. Take out the **Lysis plate**, and add **603 µL of binding buffer, 25 µL of MagBeads Suspension** to each well.

**NOTE:** Mix the binding buffer and MagBeads Suspension fully (at least 1 minute) before dispensing. Resuspend the MagBeads suspension by dispensing every 5~8 wells.

2.7 Put the **Lysis plate** back and click "**Start**" and run the procedure.

2.8 After the procedure is finished, take out the Elute plate and transfer the eluted DNA to new 1.5 mL low DNA-binding microcentrifuge tubes or PCR tubes immediately. The purified, high-quality eluted DNA is ready to use in demanding downstream applications.



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**NOTE:** It is recommended to finish qPCR testing within 24 hours, and the DNA can be temporarily stored at 2°C to 8°C. For long-term storage, it should be placed at −20°C, and you should complete the DNA testing within 7 days. A multi-channel pipette helps to transfer liquid quickly.

2.9 Take out all the plates and clean the work space with 75% ethanol.

**NOTE:** The interval between extraction experiments is recommended above 30 minutes to avoid cross contamination.



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# Appendixes

## Appendix A: FAQs

| Common Questions | Possible reasons & Actions |
|---|---|
| Do precipitates appearing in Buffer AL affect extraction efficiency? | Precipitates appearing in Buffer AL may be caused by low temperature.<br>Please dissolve the precipitates according to the instruction of part "Contents and Storage" on page 2. It has no effect on the extraction efficiency after the precipitates are completely dissolved.<br>The laboratory temperature should be kept at 20℃ to 30℃ day and night, and the temperature should not be lower than 20℃ to avoid precipitates appearing in Buffer AL. |
| How long can the eluted DNA be stored without affecting the testing results? | It is recommended to finish qPCR testing on the same day as extraction or within 24 hours. The DNA can be temporarily stored at 2°C to 8°C.<br>For long-term storage, it should be placed at −20°C, and you should complete the DNA testing within 7 days. Also, it should be frozen and thawed only once. |
| Does the kit stored at room temperature affect the extraction efficiency? | The kit has been verified and can be stored at room temperature (10-30°C) for one year. For optimal long-term stability, the MagBeads Suspension and Proteinase K are recommended to be stored at 2℃ to 8℃. |
| What could be done if the extraction efficiency of high-protein samples is poor? | For samples with high protein concentration, you can try to extend the digestion time of the samples with Proteinase K from 8 minutes to 30 minutes. |



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| | |
|---|---|
| Can samples with high cell concentration ($10^7$ cells/mL) be extracted directly for Mycoplasma detection? | It is not recommended for extraction directly.<br>An excessive amount of cellular genomic DNA and/or RNA may be extracted during extraction owing to high concentration cells, which will lead to the inhibition of qPCR and affect the detection of low-content targets.<br>Please pre-treat the samples according to the instruction of part "Prepare the samples: 2.2 Cell suspension" on page 6. |

## Appendix B: Consumables recommendation

| Consumables | Catalog Number | Manufacturer |
|---|---|---|
| 2.0 mL Low DNA-Binding Microcentrifuge Tubes | 72.695.700 | SARSTEDT |
| 1.5 mL Low DNA-Binding Microcentrifuge Tubes | 72.706.700 | SARSTEDT |



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