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# Mycoplasma DNA (qPCR) Detection Kit

**CATALOG #DK-M002, 50 Tests**

## PRODUCT INTRODUCTION

The Mycoplasma DNA (qPCR) Detection Kit is designed for qualitative detection of Mycoplasma contamination in critical biological materials, including master cell banks, working cell banks, viral seed lots, control cells, and cells for clinical treatment. Utilizing TaqMan® probe-based qPCR technology, this kit enables quantitative detection of Mycoplasma DNA in samples, delivering rapid, specific, and reliable results while covering over 150 Mycoplasma species.

It is recommended to use KACTUS Host Cell Residual DNA (Magnetic Beads) Sample Preparation Kit (Catalog No. DK-SP001) for integrated workflow solutions.

## KIT COMPONENTS

| Components | Size | Storage Conditions |
|---|---|---|
| Mycoplasma Primer & Probe MIX | 120 µL × 1 vial | -20°C, protected from light |
| 2X qPCR Reaction MIX | 700 µL × 1 vial | -20°C |
| Internal Control (IC) | 220 µL × 1 vial | |
| Positive Control （PC） | 500 µL × 1 vial | |
| DNA Diluent | 1.5 mL × 3 vials | |

## STORAGE AND SHELF LIFE

Unopened reagents maintain stability for 12 months from the date of manufacture when stored as recommended. After opening, please store the remaining reagents at -20°C.

## REQUIRED MATERIALS NOT SUPPLIED

- Real-time PCR instrument (ABI QuantStudio 3, ABI 7500, etc.), Centrifuge, Vortex.
- Optical 8-tube strip or 96-well qPCR plate (sterile, nuclease-free, and low retention), 1.5mL centrifuge tube (sterile and low retention)

## WORKFLOW

### Prepare the reagents and samples

1. **Prepare the kit.** Place all components on ice to ensure complete dissolution before use.
2. **Extract DNA from test samples.** It is recommended to extract sample DNA using KACTUS Host Cell Residual DNA (Magnetic Beads) Sample Pretreatment Kit (Catalog No. DK-SP001).
3. **Prepare the qPCR Working Mix.**
   a) Calculate the required number of qPCR reaction wells using the following formula:

i. Total wells N = (1 Non-Template Control + 1 Positive Control + Test Samples) × 2 or 3 Number of Replicates

b) Prepare the Working Mix per table below:

i. Total volume of Working Mix = 15 μL × (Total Wells N + 2)

| Components | Working Mix for single reaction |
|---|---|
| 2×qPCR Reaction MIX | 12.5 μL |
| Mycoplasma Primer & Probe MIX | 2.1 μL |
| Internal Control （IC） | 0.4 μL |
| Total volume | 15 μL |

c) **Prepare the qPCR reaction solution.** Place all the reagents on ice, prepare the qPCR reaction solution per the table below:

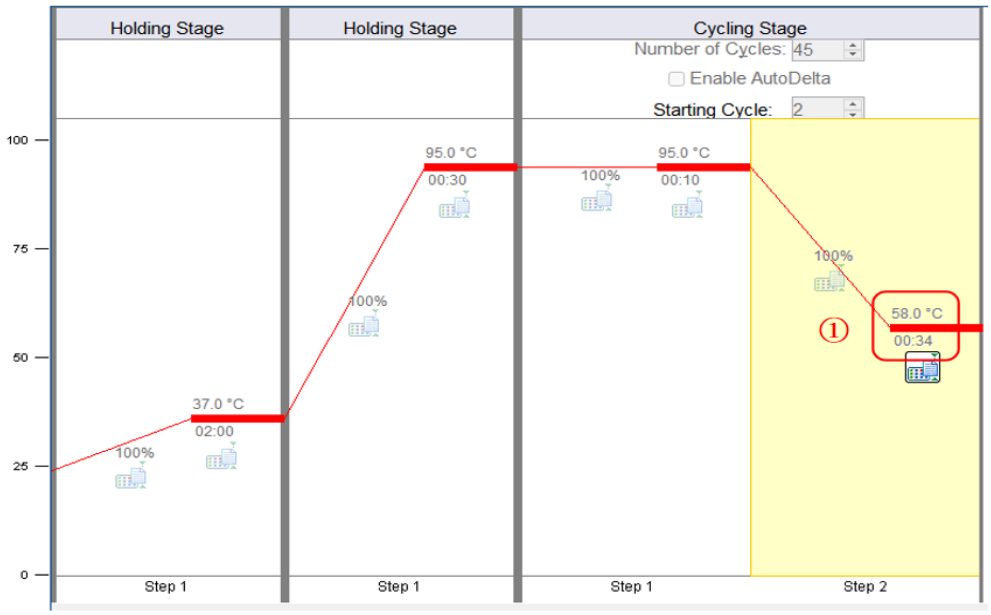
| | Sample | Positive Control (PC) | Non-Template Control (NTC) |
|---|---|---|---|
| **Template** | 10 μL Sample | 10 μL Positive Control （PC ） | 10 μL DNA Dilution |
| **Working Mix per well** | 15 μL | 15 μL | 15 μL |
| **Total Volume per well** | 25 μL | 25 μL | 25 μL |

**qPCR reaction parameters**

Using American ABI 7500 Real-Time PCR System (version 2.06) as an example:

1. Create a blank new program. Experiment Type: Quantitation-Standard Curve. Reagents: TaqMan® Reagents.
2. Use “Add New Targets” to create new probes and edit the probe names in “Target Name”. Select the desired fluorescent and quenching groups in “Reporter” and “Quencher”. “Reporter” is selected as FAM, the “Quencher” is selected as NONE, the fluorescent group of internal control (IC) is selected as VIC, and the reference fluorescence is ROX.
3. Use “Add New Samples” to add new samples and name them in the corresponding Sample name column.
4. Set the reaction parameters. 58°C for 34 s in Stage 3 is set as fluorescence collection.

| | | | | |
|---|---|---|---|---|
| **Stage 1** | Digest | Reps：1 | 37°C | 2 min |
| **Stage 2** | Pre-denaturation | Reps：1 | 95°C | 30 s |
| **Stage 3** | Cycle | Reps：45 | 95°C | 10 s |
| | | | 58°C | 34 s |




5. Click “Start Run” to perform qPCR.

## ANALYSIS

Using American ABI 7500 Real-Time PCR System (version 2.06) as an example:

1. Click “Analyze”. Check whether the shape of the amplification curve is normal.
2. Read the Ct value in the “View Well Table”.
3. NTC, PC results must meet:

| Group | FAM channel | VIC channel |
|---|---|---|
| NTC | Ct ≥ 40 or no significant amplification curve observed. | Ct < 40 with a characteristic sigmoidal amplification curve. |
| PC | Ct <40 with a characteristic sigmoidal amplification curve. | Ct < 40 with a characteristic sigmoidal amplification curve. |

4. Test sample results:

| | | |
|---|---|---|
| **Ct < 40 with a characteristic sigmoidal amplification curve.** | **Ct<40 with a characteristic sigmoidal amplification curve.** | Positive |
| | **Ct ≥ 40 or no significant amplification curve observed.** | PCR inhibition, retest. |
| **Ct ≥ 40 or no significant amplification curve** | **Ct < 40 with a characteristic sigmoidal amplification curve.** | Negative |
| | **Ct ≥ 40 or no significant amplification curve** | PCR inhibition, retest. |

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## NOTES

1. The components in the kit are stable and do not require aliquoting.
2. Prepare negative and positive samples in separate areas avoiding cross-contamination.
3. Always use clean pipette tips and avoid contamination.
4. Use the kit before expiration and do not mix different lots.
5. Thaw all components completely before use. Mix well and centrifuge briefly.
6. Follow instructions carefully for the best results.
7. Results depend on reagent quality, technique, and lab conditions.
8. Prepare enough samples for your needs.
9. For research use only. Not for medical diagnosis.

## FAQs

| Common Issues | Possible Reasons | Actions to Take |
|---|---|---|
| No Ct value | Incorrect fluorescence detection settings in the PCR program. | Ensure 58°C for 34 s in Stage 3 is set as fluorescence collection. |
| | Primer or probe degradation | Perform PAGE electrophoresis to determine if degradation had occurred. |
| | Template insufficient. | Template degradation may be caused by impurities or damaged by repeated freeze-thaw. |
| Delayed Ct | qPCR reaction components degraded or are insufficient | Verify the ROX reference signal. |
| | Inhibitors in template | |
| Negative control shows a signal | Contamination | 1. Repeat using newly prepared Working Mix.<br>2. Prepare the reaction solution in the laminar flow hood.<br>3. Separate the different experiment areas. Change to low-retention, nuclease-free tubes and pipette tips. |
| Abnormal amplification curve | 1. High concentration of template; template degraded<br>2. Evaporation or sealing issue<br>3. Bubbles in tube | 1. Dilute template. Retest with fresh reagents<br>2. Carefully transfer the reagents to avoid bubbles. |

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## RELATED PRODUCTS

| Product Name | Catalog No. |
|---|---|
| Host Cell Residual DNA (magnetic beads) Sample Preparation Kit | DK-SP001 |
| Mycoplasma DNA (qPCR) Detection Kit | DK-M002 |