SHENTEK

AdvSHENTEK Mycoplasma DetectInnova Cassette

User Guide

Version: A/0
For Research Use Only
Product No.: 16031010405

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(IMPORTANT: Please read this document carefully before experiment.)

1. Product Overview

The AdvSHENTEK Mycoplasma DetectInnova Cassette, designed for use with the AdvSHENTEK DetectInnova System, uses magnetic particle separation technology and real-time fluorescence PCR technology. The cassette is a closed, disposable system, which contains all necessary reagents for nucleic acid extraction, purification, amplification, and detection, enabling an efficient, all-in-one workflow from sample preparation to result analysis. After sampling or simplified preparation, the user pipettes Magnetic Particles and sample combined with Internal Control (IC) into the cassette, inserts the cassette into an AdvSHENTEK DetectInnova System, and starts a test. The entire test takes approximately 3 hours. For more information, please refer to the Operating Manual for the Mycoplasma DetectInnova System.

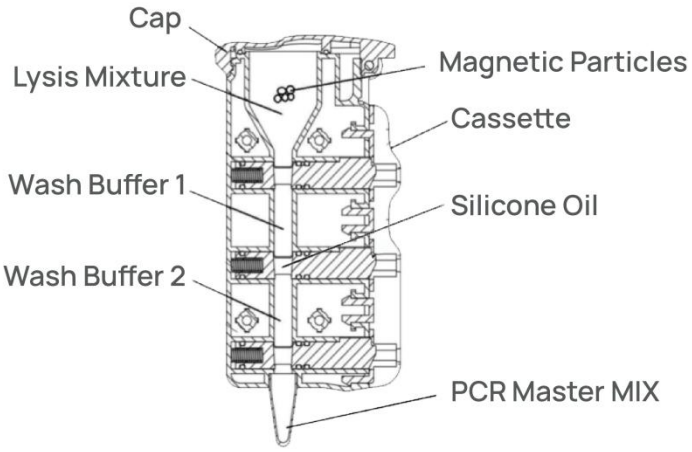

Figure 1. Schematic Diagram of the Cassette

2. Applications

The system enables the qualitative detection of adventitious agents, including *Mycoplasma, Spiroplasma, Acholeplasma, Entomoplasma, and Mesoplasma* in master and working cell banks, virus seed stocks, in-process samples, and final products, etc. It is well suited for release testing or process control testing of cell therapy samples that may contain high cell counts (up to 10^7 cells) and cell supernatants.

3. Performance Validation

The system is validated according to USP <63>, EP 2.6.7, and JP XVIII, with a detection limit of 10 CFU/mL. It can detect approximately 180 mycoplasma-related species with high specificity. Comprehensive validation studies confirm its performance in a wide range of matrices, including non-mycoplasma species, production cells, engineered bacteria, and fungi.

The closed-system design prevents contamination and ensures that false-positive results are eliminated. Internal controls are built in to monitor the efficiency of nucleic acid extraction and the entire detection process.

4. Kit Contents and Storage

WARNING: Please review the Material Safety Data Sheets (MSDSs) and adhere to handling instructions. Wear appropriate protective equipment, including eyewear, masks, gloves, and laboratory clothing.

Table 1. Kit Components and Storage Conditions

No.	Reagent	Part No.	Quantity	Storage
I	MPDI2 Cassette*	NNF002	4 Cassettes	2-8℃
II	MP Internal Control	NNA067	250 µL × 1 tube	2-8℃
	MP Positive Control	NNA068	250 µL × 1 tube	2-8℃
	Lysis Buffer	NND066	1.25 mL × 1 tube	2-8℃
	Magnetic Particles	NND065	250 µL × 1 tube	2-8℃
	Saline Buffer	NND067	500 µL × 1 tube	2-8℃
	Digestion Buffer	NND068	1 mL × 1 tube	2-8℃
	IC Buffer	NND069	1.5 mL × 1 tube	2-8℃
	Sample Buffer	NND070	5 mL × 1 bottle	2-8℃

**The cassette name on the QR code label must exactly match the corresponding test program. Scanning the QR code automatically assigns the appropriate execution program on the instrument.*

***Note:** Protect the cassette from light after opening. The kit components can be stored under the specified conditions for up to 12 months. Verify the expiration date on the label.*

5. Package

4 Cassettes/Kit

6. Kit Collection, Transport and Storage

Store the kit at 2-8ºC.

Avoid placing kits near heating or cooling vents or in direct sunlight.

All kit components should be stored and used as a complete set. Do not mix components from different lot numbers. Discard any remaining kit components once all cassettes have been used.

7. Materials Required (Not included)

- Low retention, DNase-free, sterile microcentrifuge tubes (1.5 and 2.0 mL)
- Low retention, sterile filter tips (10 µL, 100 µL, 1000 µL)
- 75% ethanol

8. Related Equipment

- AdvSHENTEK DetectInnova System

The AdvSHENTEK Mycoplasma DetectInnova Cassette is specifically designed for use with the AdvSHENTEK DetectInnova System. Upon successful login, the system interface will be displayed as shown in Figure 2. The following section provides a brief overview of the DetectInnova System to facilitate the accurate execution of the assay.

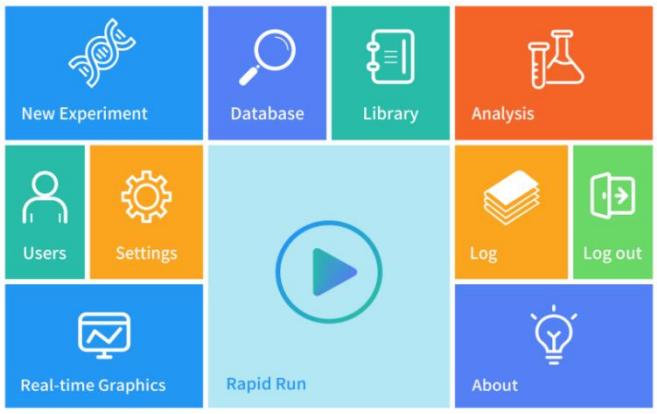

Figure 2. System Interface Displayed After Login

For detailed information on all functional buttons on the main interface, please refer to the **Operating Manual for the Mycoplasma DetectInnova Program**. Below is a brief description of the main functions:

- **Rapid Run**: Execute assays directly using Mycoplasma DetectInnova cassettes.
- **New Experiment**: Create a new experiment either via Manual Setting or by scanning QR codes on the cassettes. Since the instrument and reagents must be compatible, only authorized accounts can create new experiments. When using the AdvSHENTEK DetectInnova cassette, the standard program Mycoplasma experiment code is preloaded in the program library. Users are recommended to use Rapid Run to execute experimen. For custom program development, select Manual Setting to configure the experiment parameters.
- **Database**: View, import, export, search, and back up generated experimental data.
- **Library**: Create, edit, delete, save, and execute assay programs.
- **Analysis**: Access program titles, detection times, sample information, and experimental results for review.
- **Users**: Manage user accounts; only authorized personnel can add, edit, or delete accounts.
- **Settings**: Authorized users can configure basic instrument settings, gain settings, running modes, and perform software upgrades.
- **Log**: View operation and alarm logs, with options to export, search, and back up log files.
- **Log Out**: Safely log out from the current user account.
- **Real-time Graphics**: Monitor fluorescence curves, temperature curves, melting curves, running status, running data, and perform various operations in real time.
- **About**: View the software version, instrument model, instrument serial number, operation guide, and other system information.

9. Related Equipment (If Applicable)

- Benchtop microcentrifuge
- High-speed refrigerated centrifuge
- Heating block with block inserts, for use with 1.5 mL or 2 mL tubes
- Vortex mixer
- Pipettes (10 µL, 100 µL, 1000 µL)
- Laminar flow hood or biosafety cabinet

10. Test Procedure

10.1 Test Preparation

10.1.1 Power on a heat block to 95℃, 25℃ or 55℃ according to the sample type (please refer to 10.2.2).

10.1.2 Set the centrifuge temperature to 2-8 ℃ before use.

10.1.3 Equilibrate the Magnetic Particles (NND065) at room temperature for 10 minutes and vortex briefly before use.

10.1.4 Dilute the MP Internal Control (NNA067) 10× with IC Buffer (NND069) before use.

10.2 Sample Preparation

10.2.1 Control Samples

- Negative control samples (NCS): 1 mL of Sample Buffer (NND070).
- Positive control samples (PCS): add 10 µL of MP Positive Control (NNA068) to 1 mL of Sample Buffer (NND070).

10.2.2 Test Samples

Sample volume is recommended to be **no more than 1 mL**. Please follow the procedures in the below sections: **Samples (*Total cell count up to 10^6*)** and **Samples (*Total cell count above 10^6*)**.

- **Samples (*Total cell count up to 10^6*)**

Add 100 µL of Digestion Buffer (NND068) and 50 µL of Saline Buffer (NND067) to the sample. If the total volume is less than 1 mL, adjust to 1 mL using Sample Buffer (NND070).

- **Samples (*Total cell count above 10^6*)**

1) Heat treatment

Incubate the samples at 95℃ for 5 minutes, then cool the samples on ice for 5 minutes.

2) Cell lysis

After cooling, add Lysis Buffer (NND066) to the samples at a volume ratio of 1:10, vortex to mix well, and incubate at 25℃ for 5 minutes.

3) Removal of cell debris

Centrifuge the tubes at a minimum of 7,250 ×g for 10 seconds to pellet the cell debris, and transfer the supernatant to a new microcentrifuge tube.

Caution: Use centrifuge tubes certified for high-speed centrifugation to ensure safe operation.

4) Sample concentration

Spin the supernatant in a refrigerated centrifuge at 18,000 ×g for 10 minutes. Remove the supernatant and leave the remaining volume at approximately 100 µL. Adjust the sample volume up to 300 µL with Sample Buffer (NND070).

Caution: Use centrifuge tubes certified for high-speed centrifugation to ensure safe operation.

5) Sample digestion

Add 20 µL Digestion Buffer (NND068) and 10 µL Saline Buffer (NND067) to each tube, vortex to mix well, and spin in a microcentrifuge for 3 seconds. Incubate the mixture at 55℃ for 30 minutes.

Note: *To ensure complete digestion, vortex 2-3 times during incubation.*

Note: *For complex samples or technical testing issues, please contact our technical support team (info@shentekbio.com) for assistance.*

10.3 Sample Loading

Figure 3. Flowchart of Sample Loading

10.3.1 Make an incision in the film cover: Lift the cassette lid, then use a pipette tip to make an incision in the film cover.

10.3.2 Add Magnetic Particles (NND065) and MP Internal Control (NNA067): Use a pipette to add 10 μL of well-mixed Magnetic Particles by placing the pipette tip below the liquid surface. Then add 10 μL of diluted MP Internal Control.

10.3.3 Add the prepared sample as described in Section 10.2 into the cassette, and securely close the lid.

10.4 Run Test

10.4.1 Turn on/Power the AdvSHENTEK DetectInnova System according to the DetectInnova System User Manual. Click ‘Login’ when the login screen appears. The instrument is ready for use when the self-inspection is complete.

10.4.2 Click ‘Rapid Run’ from the main interface.

In the “Sample Info” field, enter the sample name or select the ‘ ’ button to scan the sample code using the scanner on the front cover. Next, in

the "Program Name" field, select the ‘ ’ button to scan the QR code. Please position the cassette with the QR code facing up near the scanner on the front cover for code scanning.

10.4.3 When prompted by the software, select the appropriate cassette channel, insert the cassette into the operational slot, and press firmly to ensure secure placement.

Note: *Verify the cassette orientation is correct, the QR code faces upward. Then press the lid door switch to close the door.*

10.4.4 Verify the gain (the values for each parameter must be confirmed through calibration and should remain fixed during testing), and select the button in the bottom right corner of the screen. The instrument will initiate the program, and the program interface will be displayed on the screen.

10.4.5 Review the results once the program is complete.

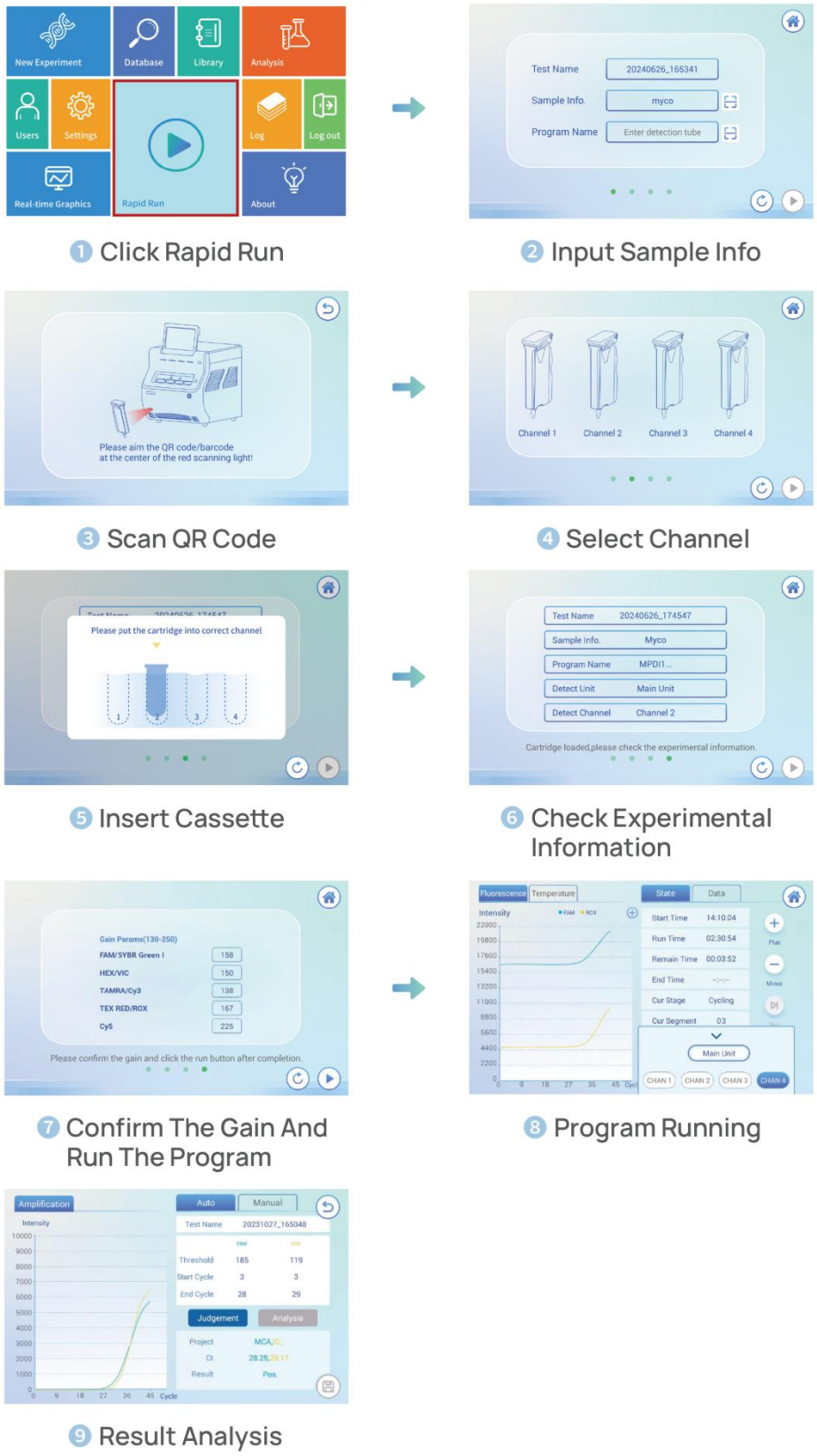

Figure 4. Program Running Graphic Scheme

Note: *It is recommended to include NCS and PCS in each run:*

1. *IC: The amplification status of the IC is used to verify that the process was operating normally and to detect any sample inhibition.*
2. *PCS: A positive result confirms the successful amplification of the positive control (PC) and proper cassettes performance.*
3. *NCS: A negative result indicates the proper functioning of the negative control (NC) and cassettes.*

Contamination may cause unexpected positive results in negative controls. If such results occur, thoroughly clean and decontaminate the workspace to eliminate potential contaminants. Contact Customer Support for further assistance if the issue persists.

11. Result Analysis

Once the program is complete, click the 'Analysis' button and select the data set analyze. Then click the  button on the right side of each row shown in Figure 5. The Ct value interpretations of test samples are displayed. Guidance for result analysis is provided in Table 2.

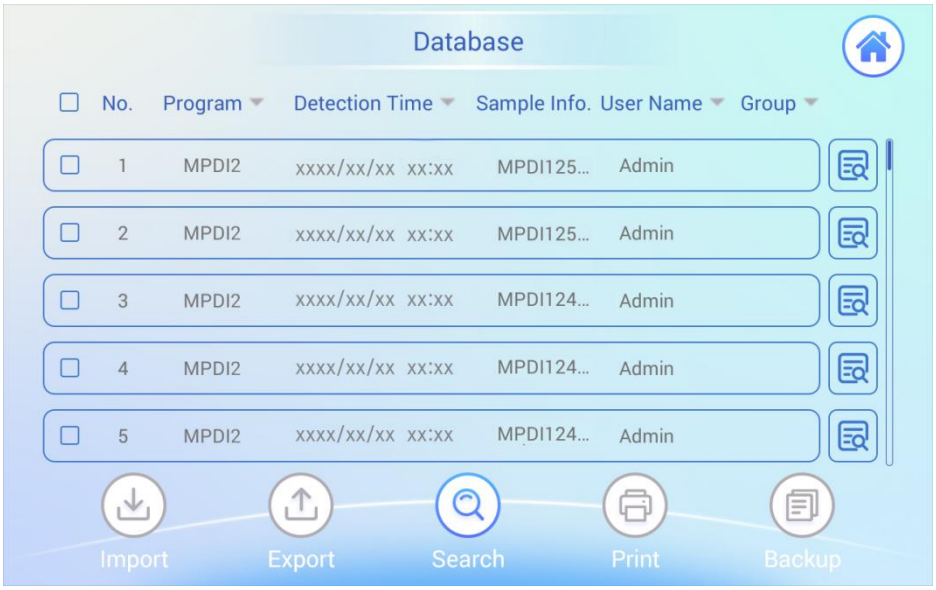

Figure 5. Database Interface

Table 2. Mycoplasma Detection Result Analysis

FAM (Target)	ROX (IC)	Result	Conclusion
Positive	Positive	Positive	Acceptable
Positive	Negative	Positive	Not Conclusive
Negative	Positive	Negative	Acceptable
Negative	Negative	Failure	Not Acceptable

12. Retests

If any of the following conditions occur, repeat the test using new cassettes.

- Abnormal IC gain: Low signal value or abnormal IC gain curve.
- No IC amplification signal: If IC was unintentionally omitted, repeat the test.

Note: *If amplification is abnormal, verify proper reagent dilution and ensure the reaction is proceeding as expected.*

- Abnormal sample amplification curve: Irregular curve.
- Other conditions: For example, liquid leakage or improper sealing of the cassette lid during operation.

13. Performance Characteristics

13.1 Limit of Detection

Eight mycoplasma strains at 10 CFU/mL and three mycoplasma strains at 5 CFU/mL were tested. For each strain, 24 assays were conducted and the detection rate was calculated. The results are summarized in the table below.

Table 3. DL (Limit of Detection) Results of the Strains

Strains	Test Conc. (CFU/mL)	Detection rate (%)	Acceptance Criteria	Conclusion
Mycoplasma orale	10	100	Detection rate at least above 95%.	Pass
Mycoplasma pneumoniae	10	100		Pass
Mycoplasma hyorhinis	10	100		Pass
Mycoplasma hominis	10	95.8		Pass
Mycoplasma fermentans	10	100		Pass
Acholeplasma laidlawii	10	100		Pass
Mycoplasma salivarium	10	100		Pass
Spiroplasma citri	10	100		Pass
Mycoplasma gallisepticum	5	100		Pass
Mycoplasma arginini	5	100		Pass
Mycoplasma synoviae	5	100		Pass

13.2 Specificity

Eighteen genomic DNAs from engineered cells and bacteria, were extracted, purified, and tested with the cassette. All test results were negative.

Table 4. Specificity Results

Types of interference	Species	Results
Natural bacteria	1. Clostridium (*Clostridium acetobutylicum, Clostridium perfringens, Clostridium sporogenes*); 2. Streptococcus (*Streptococcus pneumoniae, Streptococcus agalactiae, Streptococcus mutans*).	Negative
Engineered cells	CHO, Vero, 293T, HEK293, MDCK, Sf9, Hi5, NS0	Negative
Engineered bacteria	1. *Escherichia coli,* 2. *Pichia pastoris,* 3. *Hansenula polymorpha,* 4. *Saccharomyces cerevisiae.*	Negative

13.3 Reproducibility

13.3.1 Instrument precision

Mycoplasma orale (100 CFU) was tested on three different instruments by three analysts, with four replicates per instrument. All 12 samples were successfully detected, and both the Ct values of the samples and internal controls met the requirement of CV ≤ 15%.

13.3.2 Repeatability

Twelve assays were performed on *Mycoplasma orale* (100 CFU) by a single operator using the same instrument. All tests were successfully detected, and both the Ct values of the samples and the internal controls met the requirement of CV ≤ 15%.

14. Precautions

1. For research use only.

2. The performance of the AdvSHENTEK Mycoplasma DetectInnova Cassette has been validated exclusively on the AdvSHENTEK DetectInnova System.

3. The AdvSHENTEK Mycoplasma DetectInnova Cassette is a qualitative test and does not provide quantitative results.

4. False positive and false negative results can arise from various sources; therefore, results should be analyzed by qualified personnel.

5. Detection of mycoplasma nucleic acid depends on proper specimen collection, handling, transport, storage, and preparation. Failure to follow proper procedures in any of these steps may lead to atypical results. Improper sample collection, transport, or handling increases the risk of false positive or false negative outcomes.

6. Partial PCR inhibition may occur due to high cell densities and potential inhibitory factors.

7. The environment must be kept clean before and after the experimental tests to prevent mycoplasma contamination.

8. There is a risk of false positive results due to contamination of organisms, including nucleic acids, vaccine material, amplified product or non-specific signals in the assay.

9. It is recommended to designate separate areas for negative, positive, and amplification zones with clear markings. Equip each area with independent equipment, reagents, and consumables to avoid cross-contamination. This minimizes unnecessary movement and contamination risks.

10. Centrifuge the tube before opening to reduce the risk of contaminating gloves or pipettes with reagents. Used tips and liquid waste must be disinfected, then discarded in a designated container, and, if necessary, shipped off-site.

11. Remove the cassette immediately after the test is completed, seal it in a designated plastic bag, and place it in the appropriate disposal container. Do not open the cover of the cassette.

12. Do not use if the cassette is damp or the lid seal is damaged.

13. Each cassette is for single use only. Do not reuse.

For further inquiries, please contact us for technical support.

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Support & Contact

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