Five Infectious Diarrhea Bacteria Nucleic Acid Test Kit

(PCR-Fluorescent Probe Method)

1【Product Name】

Five Infectious Diarrhea Bacteria Nucleic Acid Test Kit (PCR-Fluorescent Probe Method)

2【Specification】

10 tests/kit (10200159A), 50 tests/kit (10200159B)

3【Intended Use】

This reagent kit is designed for the detection of DNA from *Campylobacter jejuni*, *Campylobacter coli, Vibrio parahaemolyticus, Salmonella,* and *Shigella* in rectal swab samples collected from individuals, serving as an auxiliary diagnostic tool for human diarrheal bacterial infections.

The test results of this product are for clinical reference only and should not be used as the sole criterion for clinical diagnosis. It is recommended to comprehensively analyze the patient's condition by integrating clinical manifestations, other laboratory tests, and relevant diagnostic criteria.

4【Principle of the Procedure】

The Five Infectious Diarrhea Bacteria Nucleic Acid Test Kit (PCR-Fluorescent Probe Method) is an automated in vitro diagnostic test that uses real-time fluorescent PCR for the qualitative detection of *Campylobacter jejuni*, *Campylobacter coli, Vibrio parahaemolyticus, Salmonella,* and *Shigella*. Primers and probes target conserved regions of each pathogen to enable specific amplification and fluorescent detection.

This assay is performed on the iFIND® Full-Automatic Nucleic Acid Detection and Analyzer system, which automates and integrates sample purification, nucleic acid extraction, amplification, and detection. The iFIND system consists of an instrument, a PAD controller with pre-installed software, and pre-configured testing workflows. The system uses single-use, self-contained iFIND cartridges that contain the ultrasonic buffer and amplification reagents, and function as the reaction chamber for nucleic acid extraction and PCR.

The assay is designed for rectal swab samples. Each cartridge contains an internal control to monitor sample processing efficiency and the presence of PCR inhibitors. During testing, the iFIND instrument measures real-time fluorescence and generates amplification curves for qualitative identification of each target pathogen.

All reactions occur within the sealed cartridge. Because all processing steps are fully contained and isolated within the cartridge, the risk of cross-contamination between samples is effectively minimized.

5【Reagents】

5.1 Materials Provided

The Five Infectious Diarrhea Bacteria Nucleic Acid Test Kit contains sufficient reagents to process 10 (10200159A), or 50 (10200159B) samples respectively.

Table 1. Components of Kit A

Cartridges	**10 per kit**	**50 per kit**
1. Bead 1, Bead 2, and Bead 3 (freeze-dried)	1 of each per cartridge	1 of each per cartridge
2. Wash Reagent	3 mL per cartridge	3 mL per cartridge
3. Sonication Reagent	3 mL per cartridge	3 mL per cartridge
Sample Preservation Solution	2 mL×10 tubes	2 mL× 50 tubes
Disposable Transfer Pipettes	10 per kit	50 per kit
Disposable Sampling Swab	10 per kit	50 per kit
Instructions for Use	1 per kit	1 per kit

Note: The disposable transfer pipettes and swab in Kit A are externally sourced, and their shelf life is indicated on their labels.

5.2 Materials Available but Not Provided

Table 2. Contents of Kit B

Positive Control	**Components**
Lyophilized powder of standard strains containing *Campylobacter jejuni, Campylobacter coli, Vibrio parahae-molyticus, Salmonella, Shigella* and internal standard gene fragments	1 test per kit
Negative Control	1 test per kit
Lyophilized powder containing internal standard gene fragments	
Diluent	2 tubes per kit
Nuclease-free water 2 mL	

Kit B provides positive and negative controls designed to con-

firm proper kit function and to assess the testing environment and operator performance. The controls are recommended to be used in the following situations:

1. Initial verification: Run both positive and negative controls when using the kit for the first time.
2. Troubleshooting: If questionable or inconsistent results are obtained, run both controls to evaluate the kit, the environment, and operator handling.
3. Environmental monitoring: If contamination of the testing environment is suspected, run both controls to verify result reliability.

6【Storage, Transport and Handling】

1. Kit A should be stored and transported at 2℃ to 28℃, protected from light, and free from freezing. The kits have a shelf life of 12 months when stored under the recommended storage conditions.
2. Production date and expiration date: refer to the outer packaging.

7【Applicable Instrument】

The Kit is compatible with the Full-Automatic Nucleic Acid Detection and Analyzer (Models: iFIND S2, iFIND S4, iFIND S8; Medical Device Registration Certificate No.: 20233221927) manufactured by RocGene (Xuzhou) Technology Co., Ltd.

8【Specimen Collection, Handling and Storage】

8.1 Specimen Type

Rectal swab samples.

8.2 Specimen Collection

Take the lateral decubitus position or the knee-thoracic position. Soak the provided swab in normal saline, then insert it 3-4 cm into the rectum along the anal wall. Gently rotate the swab twice, then slowly withdraw it. Immediately place the swab into the provided preservation solution tube. Alternatively, normal saline, Cary-Blair, or an eSwab system can be used (these are not provided in the kit).

8.3 Specimen Storage

1. Maintain recommended storage conditions to preserve specimen integrity.
2. Test specimens as soon as possible after collection.
3. Storage conditions:
    - Room temperature (10–30 °C): 6 hours
    - Refrigerated (2–8 °C): 12 hours
    - Frozen (−20 ± 5 °C): 6 months
4. Frozen specimens must be completely thawed and mixed before use.
5. Avoid more than three freeze–thaw cycles.

9【Assay Procedure】

9.1 Specimen Pre-processing

Mix the sample thoroughly by shaking the tube about 20 times or vortex for approximately 10 seconds. If the fecal content is excessive, dilute the sample with the preservation solution until at least 0.75 mL of clear supernatant is available for pipetting before testing.

9.2 Cartridge Preparation

1. Open the cartridge lid and remove the aluminum seal.
2. Using the provided pipette, transfer 0.75 mL of the supernatant (prepared according to Section 9.1) into the sample chamber (large opening) of the cartridge.

Note: Dispense the sample slowly along the chamber wall to avoid bubbles.

3. Close the cartridge lid firmly.

Note: If repeat testing is needed, store the remaining sample at 2–8 °C for up to 12 hours.

10【Run the Test】

1. Power on the RocGene iFIND® System.
2. Log in with your username and password.
3. Wait for the system to complete the self-check. Once finished, click **"OK"** to enter the **"Running"** window and select the desired instrument unit.
4. Scan or manually enter the **Cartridge ID**; When the Cartridge ID is entered correctly, the corresponding test project will be selected automatically.
5. Scan or manually enter the **Sample ID.** The Sample ID will be inserted into the middle of the default Experiment Name.

***Example*:** *If the default name is 20250907-165208 and Sample ID 888888 is entered, the name becomes 20250907-888888-165741.*

6. By default, the Experiment Name is generated from the date and time the system was powered on. Edit if needed; the name will be linked to the test results in the **"Analyze"** window.
7. Click **"Open Door"** to open the module.
8. Insert the cartridge with the amplification tube facing the inside of the module.
9. Close the door.
10. Click **"Start Run"** to initiate the assay.
11. During the run, the green indicator flashes. When complete, it remains solid.
12. After the run finishes, click **"OK"** in the prompt box.
13. Navigate to the **Analyze** window to review and confirm test results.

11【Quality Control】

Table 3. Fluorescent Channels and Targets

Channel	Target
ATTO	*Salmonella*
FAM	*Campylobacter coli*
VIC	*Campylobacter jejuni*
ROX	*Vibrio parahaemolyticus*
CY5	*Shigella*
CY55	Internal Control

Each test includes an internal quality control (IC), which verifies that the cartridge and test process are functioning

properly. If the quality control criteria are not met, the test results shouldn't be interpreted.

Internal Control (IC):

The internal control confirms that:

- the sample was added correctly,
- reagents are functioning as intended,
- the procedure was performed correctly, and
- no interfering substances prevented amplification.

External Controls:

External quality controls (see Section 5.2) are available but not provided. They may be used in accordance with local, state, or federal regulations, as applicable.

12【Cut-off Values】

Based on clinical sample testing, the positive cutoff values for *Campylobacter jejuni*, *Campylobacter coli, Vibrio parahaemolyticus, Salmonella,* and *Shigella* were established using the ROC curve method. A result is considered positive when the Ct value is ≤ 40 in the corresponding detection channel.

13【Interpretation of Results】

When the instrument is operating normally and the internal control test results meet all requirements, test sample results are analyzed and interpreted. Results are automatically generated by the instrument.

Table 4. Interpretation of Results

Result	Interpretation of results & action
Positive for *Campylobacter coli*	*Campylobacter coli* is present in the sample.
Positive for *Campylobacter jejuni*	*Campylobacter jejuni* is present in the sample.
Positive for *Shigella*	*Shigella* is present in the sample.
Positive for *Vibrio parahaemolyticus*	*Vibrio parahaemolyticus* is present in the sample.
Positive for *Salmonella*	*Salmonella* is present in the sample.
Negative	There is no *Campylobacter jejuni, Campylobacter coli, Vibrio parahaemolyticus, Salmonella,* and *Shigella* genes or the content is below the detection limit in the sample.
Result Requires Repeat	Repeat test using the original sample. If the repeat result remains invalid, consider collecting a new specimen.

14【Limitations】

1. The test results of this product are for clinical reference only and should not be used as the sole criterion for clinical diagnosis.
2. The primer or probe binding region is a conserved region of the pathogen, and mutations rarely occur, but genetic mutations in the epidemic process of the pathogen cannot be ruled out, resulting in false negatives.
3. Inappropriate sample collection, transportation, handling, experimental procedures, or experimental conditions, may all lead to false negative and/or false positive results.
4. Only common mutations have been validated in clinical trials, Rare or novel strains have not been clinically validated.
5. Contamination by microorganisms and amplification products may cause erroneous results. Strict attention should be paid to laboratory division and the PCR standard laboratory operation method should be followed.

15【Analytical Performance Characteristics】

1. **Limit of Detection (LoD)**
   500 CFU/ml.
2. **Analytical specificity**
   No cross reactivity with other enteric pathogens.

16【Warnings and Precautions】

1. For in vitro diagnostic use only. Please read the product manual carefully before operation.
2. All samples for detection should be handled as potentially infectious. Wear laboratory coats, protective disposable gloves and change the gloves often to avoid cross-contamination between samples. Handling of samples and waste must meet relevant requirements outlined in local, state and national regulations.
3. This kit does not contain active substances of human or animal origin.
4. Product performance is verified only for sputum sample and the sample collection and processing method described in section 8【Specimen Collection, Handling and Storage】. If other sample types or sample collection and processing methods are used, product performance cannot be guaranteed.
5. The kit still has a certain amount of biological load and pollutants after use, handle the reagent with appropriate precautions and good laboratory practice.
6. Note: Improper operation during the storage, transportation and use of the reagent may affect the test results. For example, improper storage and transportation, sample collection, sample processing and test process are not standardized. Please strictly follow the instructions.

17【Key to symbols】

Symbols	Title	Symbols	Title
CE	CE Marking	IVD	In vitro diagnostic medical device
2℃ 28℃	Temperature Limitation		Keep away from sunlight
	Do not use if package is damaged		Do not re-use
	Consult Instruction for Use		Caution
	Manufacturer	EC REP	Authorized representative in the European Community/

Symbols	Title	Symbols	Title
			European Union
REF	Reference	/	/

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