Instruction Manual：Kaneka Nucleic Acid Chromatography Carbapenemase Gene Detection Kit

Precautions for use

- **Wear protective equipment when using this product, (rubber gloves, protective glasses, mask, etc.).**
- **It is the customer's responsibility to judge and use the results obtained with this product. The Company shall not be liable for any damage or loss caused by determining or using the results, whether directly or indirectly.**
- **Please verify the validity of the result obtained by the operation procedure not described in this instruction manual.**
- **This product is a research reagent and may not be used for the purpose of diagnosing disease or supporting it.**

1. Kit Overview

This kit is for detecting five representative carbapenemase genes (IMP, VIM, NDM, KPC, OXA48) from bacterial suspension or bacterial culture based on multiplex PCR and nucleic acid chromatography.

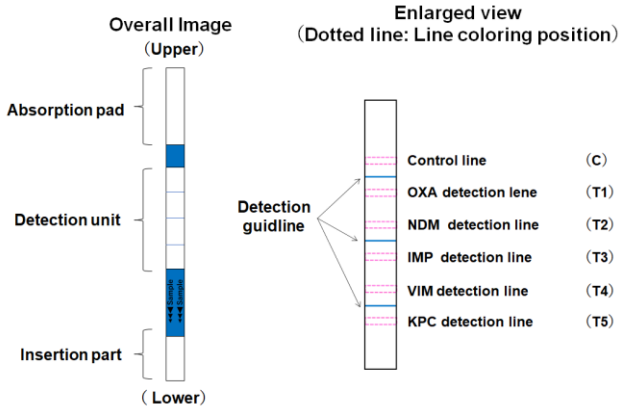
2. Kit composition/Storage condition

Component	Contents (20 tests)	Storage Temp.	Expiration Date
Test strip	20 strips	Room Temp. (2-30℃)	Listed on label
Chase buffer	4 mL×1		
PCR mix※1	200 μL×1	Frozen (Below -20℃)	
Primer mix	100 μL×1		

※1. DNA Polymerase dU plus dNTP mixture (dATP, dCTP, dGTP, dUTP)、UNG（Uracil DNA Glycosylase）included.

3. Detection Principle

The target gene is amplified by multiplex PCR using primers that specifically amplify the carbapenemase gene. The amplified product is spread on a test strip and the carbapenemase genotype is determined from the line coloring pattern. Line coloring can be visually confirmed, and a device such as a gel imager or a real-time PCR machine is not required to evaluate the result.



4. How to Use

4.1 Equipment and device required in addition to this kit

- Micropipette and tip with filter for micropipette
- Thermal cycler (The performance of this product has been confirmed by LifeECO Thermal Cycler (Bioer Technology)
- Heat block (can be substituted with heat block)
- 0.2 mL Tube (for PCR)
- Desktop centrifuge

4.2 Preparation of DNA extract

Use a commercially available DNA extraction kit (Kaneka Easy DNA Extraction Kit Version 2, DNeasy Blood & Tissue Kit - QIAGEN, etc.) to extract DNA using a strain suspected of producing carbapenemase that was isolated by a selective separation medium. In addition, purified DNA prepared using the phenol/chloroform method or DNA extracted by the heat extraction method can also be used as a sample.

Example) Method using Kaneka Easy DNA Extraction Kit Version 2

(1) Suspend the colonies obtained by the separation culture in sterile physiological saline, and use the McFarland turbidimetric method to prepare the turbidity standard solution No. 0.5-2.

(2) Transfer 15 µL of the suspension to a 0.2 mL tube (for liquid culture, use the stock solution of the culture medium as the bacterial solution), mix with 100 µL of Reagent A, and heat at 98°C for 10 minutes in a heat block.

(3) After cooling with ice, add 15 µL of Reagent B and pipette.

Example) Method by heat extraction

(1) Transfer 100 µL of the colony suspension or the stock solution of liquid culture to a 0.2 mL tube, and heat it at 98℃ for 10 minutes in a heat block (or thermal cycler).

4.3. Preparation of PCR solution

(1) Thaw the PCR mix and primer mix in advance*2.

(2) Prepare a master mix by dispensing and mixing the required number of minutes in a 0.2 mL tube for preparing the master mix at a ratio of 10 µL of PCR mix, 5 µL of primer mix, and 3 µL of sterilized distilled water*3.

(3) Add 2 µL of the DNA extract prepared in 4.2. to a new 0.2 mL tube.

(4) Add 18 µL of master mix to the 0.2 mL tube of (3) and mix by pipetting.

*2 Thaw and mix the PCR mix and primer mix thoroughly. In particular, the PCR mix may show a white precipitate when thawed. In this case, use the pipette to completely dissolve the precipitate.

*3 After preparation, cool on ice to prevent enzyme inactivation and non-specific amplification.

4.4 PCR

Set a 0.2 mL tube in the thermal cycler and perform the amplification reaction under the PCR conditions in the table below*4.

Temp.（℃）	Time（Sec）	Cycle
25	300	1
95	180	1
94	20	35
58	20	
72	45	
72	300	1

*4. If the detection with the test strip is not performed immediately after completion of the PCR reaction, it is recommended to store the PCR reaction solution in a frozen state. Thaw the frozen PCR reaction solution completely at room temperature before performing detection with a test strip.

4.5. Detection by test strip

(1) After completion of the reaction, remove the PCR tube, open the lid, and add 160 µL of chase buffer.

(2) Hold the upper part of the test strip and remove it from the case*5, and insert the test strip insertion part (the side with the letters "◂◂◂Sample") firmly to the bottom of the PCR tube*6 (PCR solution will begin reacting.)

(3) After 10 minutes of reaction, visually check the line coloring pattern and evaluate.

*5. When handling the test strip, be sure to wear rubber gloves and always hold the absorbent pad on the top. Do not directly touch the insertion part or the detection part of the test strip.

*6. When inserting the test strip into the PCR tube, pay careful attention to contamination due to scattering of the PCR solution.

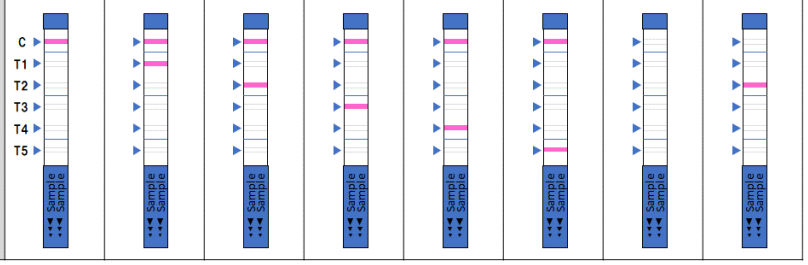
5. Results

Negative: Only the control line (C) is colored pink to magenta.

Positive : In addition to the control line (C), the detection lines (T1 to T5) are colored pink to magenta.

Invalid : Control line (C) is not colored (the figure below is one of an example) *9.

Result	Negative	Positive					Invalid	
		OXA48	NDM	IMP	VIM	KPC		
Color patterns	Only C	C+T1	C+T2	C+T3	C+T4	C+T5	C none	C none



*7. If the control line is not colored, there is a possibility of erroneous operation or malfunction of the equipment. Therefore, retest from the preparation of the DNA extract in Section 4.2.

*8. If the DNA extract contains a large amount of contaminants, it may interfere with the PCR and lighten the line coloring. In that case, it may be improved by diluting the DNA extract.

*9. If the environmental temperature at the time of detection is extremely low (outdoors in winter, etc.), the line coloring may become light, so please use it indoors air-conditioned at 15°C or higher.

6. Precautions for use

- Strictly adhere to the storage conditions and expiration dates described in this instruction manual.
- The specifications of this product are subject to change without notice.
- Follow the instruction specified by the manufacturer for the equipment and devices to be used.
- If the test strip is left for a long time in a moisture-containing condition, the detection performance may deteriorate. After opening the test strip, keep the container lid tightly closed and store it carefully.
- Change the tip of the micropipette after each use and be careful of contamination. Also, use a micropipette tip with a filter.
- Before and after using this product, clean the test bench with 0.55% sodium hypochlorite aqueous solution, DNA remover, ultraviolet (UV), etc.
- To prevent misjudgment due to contamination, we recommend that each step of DNA extraction, PCR solution preparation, and test strip detection be performed in separate areas.
- Before opening the tube containing the reagents and DNA extract, spin down with a tabletop centrifuge (to avoid scattering of the contents).
- If contamination is confirmed, clean the equipment and devices to be used according to the method specified by the manufacturer.
- When discarding the used test strip and PCR solution, be careful not to touch the test strip insertion section, the detection section and the PCR solution directly, and put them in a vinyl bag etc. to prevent the PCR solution from scattering. Please dispose of it.
- When discarding this product, PCR solution, DNA extraction solution, etc., dispose in consideration of hygiene and environment in accordance with the regulations on waste in the area concerned and the regulations of the facility concerned.
- The performance of this product has been confirmed by LifeECO Thermal Cycler of Bioer Technology. When using other models, the line coloring of the test strip may become unclear.

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