Code No. KN-T10C815
1st edition (April 2021)

Instructions for use: KANEKA DNA Chromatography MABC/*erm* (41)

Precautions for use

- **Wear personal protective equipment (such as rubber gloves, safety glasses, and masks) before using KANEKA DNA Chromatography MABC/*erm* (41).**
- **The results obtained from KANEKA DNA Chromatography MABC/*erm* (41) should be evaluated and used at your own discretion. Kaneka Corporation is not responsible, whether directly or indirectly, for any damages or loss which may be caused by the evaluation or use of the obtained results.**
- **It is your responsibility to verify the validity of any detection results obtained using an operating procedure that is not indicated in these instructions for use.**
- **KANEKA DNA Chromatography MABC/*erm* (41) is a set of reagents intended for research applications and is not to be used to diagnose, or help to diagnose, a disease.**

1. Product description

KANEKA DNA Chromatography MABC/*erm* (41) (research reagents) is intended to identify three subspecies of *Mycobacteroides abscessus* complex (MABC) (*M. abscessus* subsp. *abscessus* [*M. abscessus*], *M. abscessus* subsp. *bolletii* [*M. bolletii*], and *M. abscessus* subsp. *massiliense* [*M. massiliense*]) and two genetic types (T28C, full-length*1) related to *erm* (41) in bacterial suspension or bacterial broth medium based on the multiplex PCR and nucleic acid chromatography techniques.

*1. T28C strain: A strain in which nucleotide 28 of *erm* (41) is C. Full-length: A strain that keeps the full length of the *erm* (41) gene intact without any partial deletions (nucleotides 64–65 and 159–432 are typically deleted). KANEKA DNA Chromatography MABC/*erm* (41) was co-developed by Dr. Yoshihiko Hoshino, the National Institute of Infectious Diseases, and Kaneka Corporation. Its detection capability was published in EBioMedicine (2021 Feb; 64:103187).

2. Kit structure/storage conditions

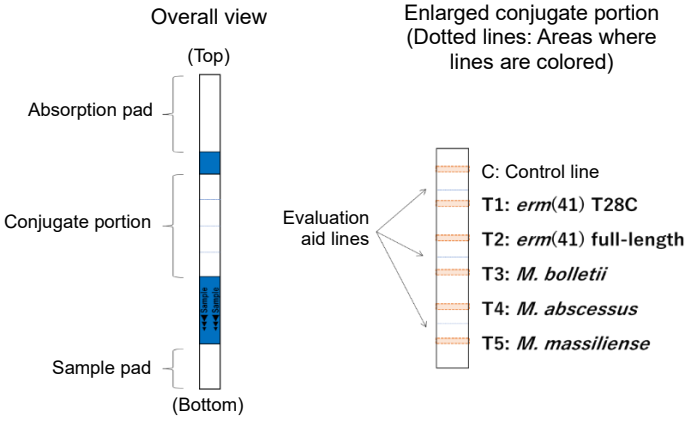
Component	Volume (20 tests)	Storage temperature	Expiration date
Test strips	20 strips	2 to 30˚C	Indicated on the label
Detection buffer	3.2 mL x 1 vial		
Results card	20 pcs.		
PCR mix*2	200 µL x 1 vial	-20˚C or less	
Primer mix	100 µL x 1 vial		

*2. The PCR mix contains heat-resistant DNA polymerase, dNTPs, dUTP, and uracil DNA glycosylase (UNG).

3. Detection principle

DNA regions that have been specifically stored in each item are amplified with multiplex PCR by using primers that specifically amplify each item. The amplified product is spread on a test strip, and MABC subspecies and *erm* (41) genetic types are determined based on the colored line pattern. The colored lines are checked visually, so no equipment is needed to evaluate the result (such as a gel imaging equipment or real-time PCR system).

<Names of test strip parts>



4. How to use

4.1. Instruments and equipment that are necessary but not included in KANEKA DNA Chromatography MABC/*erm* (41)

- Micropipette and micropipette tips with filters
- Thermal cycler (recommended: LifeECO Thermal Cycler/Bioer Technology)
- Heat block (can be substituted with a thermal cycler)
- 0.2 mL tubes
- Desktop centrifuge
- KANEKA Microbial DNA Extraction Reagent (sold separately)

4.2. Preparation of DNA extract

Applicable specimens

Suspension of culture grown on a solid medium or in a liquid medium or broth medium.

- Equivalent to McFarland No. 1 (approximately 10^8 cfu/mL) or culture positive liquid medium
- Bacterial suspension prepared with colonies in sterile distilled water so that they are equivalent to McFarland No. 1

DNA extract reagent

"KANEKA Microbial DNA Extraction Reagent" (sold separately)

<For liquid medium>

(1) Transfer 20 µL of liquid medium equivalent to McFarland No. 1 to a 0.2 mL tube, mix with 20 µL of solution A of KANEKA Microbial DNA Extraction Reagent, then heat the mixture at

98˚C for 10 minutes on a heat block or thermal cycler.

(2) After cooling the mixture to room temperature, add 80 µL of solution B of KANEKA Microbial DNA Extraction Reagent, then pipette the tube.

<For colonies>

(1) Suspend colonies in sterile distilled water[*3] so that they are equivalent to McFarland No. 1.

(2) Transfer 20 µL of bacterial suspension to a 0.2 mL tube, mix with 20 µL of solution A of KANEKA Microbial DNA Extraction Reagent, then heat the mixture at 98˚C for 10 minutes on a heat block or thermal cycler.

(3) After cooling the mixture to room temperature, add 80 µL of solution B of KANEKA Microbial DNA Extraction Reagent, then pipette the tube.

*3. When collecting colonies, be careful not to scrape up the solid medium with the colonies.

4.3 Preparation of PCR reaction solution

(1) Thaw the PCR mix and primer mix beforehand. [*4]

(2) Dispense and mix the PCR mix and primer mix at a ratio of 10 µL and 5 µL, respectively, into 0.2 mL tubes to prepare the master mix for the number of tubes necessary.

(3) Add 5 µL of the DNA extract prepared in 4.2 into a new 0.2 mL tube.

(4) Add 15 µL of the master mix into the 0.2 mL tube in (3) and mix the mixture by pipetting the tube. [*5]

*4. The PCR mix and primer mix should be mixed thoroughly before dispensing. In particular, the PCR mix may show a white precipitate after thawing. In such a case, completely dissolve the precipitate before use.

*5. Once prepared, the mixture should be kept in a place where the temperature does not rise, such as on ice, in order to prevent the deactivation of enzyme and non-specific amplification.

4.4. PCR reaction

Load the 0.2 mL tube on the thermal cycler and perform an amplification reaction under the conditions listed below.

<PCR conditions>

Temperature (˚C)	Time (sec)	Cycle
25	300	1
94	60	1
94	5	35
65	10	
72	15	

4.5. Detection with test strips*[6]

(1) Place a test strip on a results card (optional). *[7]

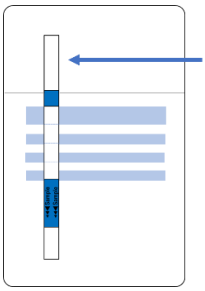
(2) After the reaction is complete, remove the PCR tube, open the lid, separate 5 µL of the amplified product, and add the product onto the sample pad of the test strip. *[8]

(3) Similarly, add 70 µL of detection buffer on the sample pad.

(4) At 10 minutes after spreading, visually check the pattern of the colored lines and evaluate the result.

*6. Wear rubber gloves or similar protective gloves when handling test strips, and always hold the absorption pad (the upper part of the test strip). Do not touch the sample pad or conjugate portion of the test strips.

*7. This step may be omitted. Placing a test strip on a results card for detection makes it easier to evaluate the pattern of the colored lines. Place a test strip if necessary, as shown in the figure below.



Place the absorption pad of the test strip into the depressed portion of the results card, with the printed face up. (Optional)

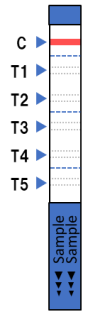
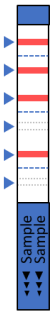
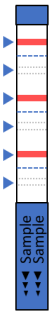
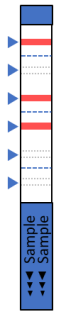
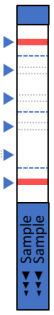
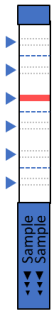
*8. When applying the amplified product to a test strip, use caution to prevent contamination caused by dispersed PCR reaction solution. Use the test strips indoor where the temperature is controlled to 15˚C or higher because the colored lines may appear light if the environmental temperature is significantly low (such as outdoors in the winter) while the amplified product is being spread.

5. Evaluation of result

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

Positive: In addition to the control line (C), a test line or test lines (T1–T5) are colored.

Invalid: The control line (C) is not colored. *[9]

Example of result	Negative	Positive*10				Invalid (example)	
		M. abscessus *erm*(41) [full, T28C]	*M. abscessus* *erm*(41) [full, T28]	*M. bolletii* *erm*(41) [full, T28]	*M. massiliense* *erm*(41) [truncation, T28]		
Coloration pattern	C only	C+T1,2,4	C+T2,4	C+T2,3	C+T5	No colored line on C	No colored line on C
							

*9. The control line is always colored. If it is not colored, the control may have not been amplified properly. Retest the specimen.

*10. These are examples of typical coloration patterns. *erm* (41) [full]: A strain that keeps the erm (41) gene intact without any partial deletions. *erm* (41) [truncation]: A strain with *erm* (41) that has partial deletions (nucleotides 64-65 and 159-432 are typically deleted).

6. Precautions for use

- Always follow the storage conditions and expiration date indicated in these instructions for use.
- Wear personal protective equipment (such as protective gloves, safety glasses, and masks) before using KANEKA DNA Chromatography MABC/*erm* (41).
- The specifications of KANEKA DNA Chromatography MABC/*erm* (41) are subject to change without prior notice.
- Follow the instructions specified by the manufacturers of the instruments, equipment, and reagents.
- The results obtained from KANEKA DNA Chromatography MABC/*erm* (41) should be evaluated and used at your own discretion. Kaneka Corporation is not responsible, whether directly or indirectly, for any damages or loss which may be caused by the evaluation or use of the obtained results.
- You are responsible for verifying the validity of a procedure that is not indicated in these instructions for use and of any detection results obtained from specimens.
- Exposing the test strips to humidity for a long period of time may lower their detection capability. Store the open container with the lid closed tightly, away from humidity.
- Replace the micropipette tips after each use in order to prevent false results due to contamination. Use micropipette tips with filters.
- It is recommended that the DNA extraction, preparation of the PCR reaction solution, and detection with test strips be done in different laboratories. If it is not possible to do so, separate the work benches

or work areas within one laboratory.

- Before opening tubes containing reagents, DNA extract, etc., spin down the tubes in a desktop centrifuge or other equipment (to prevent the content from dispersing or the reagents on the lid from transferring to the operator's fingers).
- Clean the instruments and equipment being used, such as the micropipette, periodically with 0.1% sodium hypochlorite or a commercially available DNA removal agent.
- If contamination has occurred, decontaminate the work environment with 0.55% sodium hypochlorite (or a commercially available DNA removal agent), ultraviolet (UV) light, or other methods.
- To dispose of used test strips and amplification reaction solution, place them in a plastic bag or similar container, making sure not to touch the conjugate portion.
- Dispose of the specimens after DNA extraction in accordance with the regulations concerning waste in your region and institution while considering hygiene and environmental factors.
- KANEKA DNA Chromatography MABC/*erm* (41) is optimized for LifeECO Thermal Cycler of Bioer Technology. Using other models may render unclear results.
- KANEKA DNA Chromatography MABC/*erm* (41) is a set of reagents intended for research applications and is not to be used to diagnose, or help to diagnose, a disease.

<Contact information>

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