






















Petrifilm™

6410/6411/6416/6443

Product Instructions

-  (EN) Coliform Count Plate
-  (FR) Test pour la numération des Coliformes
-  (DE) Coliforme Zählplatte
-  (IT) Piastra per il conteggio dei coliformi
-  (ES) Placa para recuento de coliformes
-  (NL) Coliform Telplaat
-  (SV) Odlingsplatta för koliformer
-  (DA) Coliform Tælleplade
-  (NO) For koliforme bakterier
-  (FI) Koliformien kasvatusalusta
-  (PT) Placa para Contagem de Coliformes
-  (EL) Πλακίδιο Καταμέτρησης Κολοβακτηριδίων
-  (PL) Płytko do oznaczania liczby bakterii z grupy coli
-  (RU) Тест-пластина для подсчета колиформных бактерий
-  (TR) Koliform Sayım Plakası
-  (JA) 大腸菌群数測定用プレート
-  (ZH) 大肠菌群测试片
-  (TH) แผ่นอาหารเลี้ยงเชื้อสำหรับนับจำนวนโคลิฟอร์ม
-  (KO) 대장균군 측정용 플레이트



Coliform Count

Product Instructions

Coliform Count Plate

Product Description and Intended Use

The 3M™ Petrifilm™ Coliform Count (CC) Plate is a sample-ready-culture medium system which contains modified Violet Red Bile (VRB) nutrients, a cold-water-soluble gelling agent, and a tetrazolium indicator that facilitates colony enumeration. 3M Petrifilm CC Plates are used for the enumeration of coliforms in the food and beverage and bottled water industries. 3M Petrifilm CC Plate components are decontaminated though not sterilized. 3M Food Safety is certified to International Organization for Standardization (ISO) 9001 for design and manufacturing. 3M Petrifilm CC Plate has not been evaluated with all possible food products, food processes, testing protocols or with all possible microorganism strains.

The United States Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM)⁷ define coliforms as Gram-negative rods, which produce acid and gas from lactose fermentation. Coliform colonies growing on the 3M Petrifilm CC Plate produce acid, which causes the pH indicator to deepen the gel color; gas trapped around red colonies indicates coliforms.

ISO defines coliforms by their ability to grow in method-specific, selective media. ISO method 4832¹, enumerating coliforms by colony-count technique, defines coliforms as acid producers on VRB with lactose (VRBL) agar. On 3M Petrifilm CC Plates these acid-producing coliforms are indicated by red colonies with or without gas production. ISO method 4831², enumerating coliforms by the most probable number (MPN) method, defines coliforms by their ability to grow and produce gas from lactose in a selective broth. On 3M Petrifilm CC Plates these coliforms are indicated by red colonies associated with gas.

For bottled water samples, confirmed coliform colonies are indicated by red colonies associated with gas on 3M Petrifilm CC Plates. Red colonies without closely associated gas bubbles may be coliforms and should be subcultured and tested with appropriate confirmation methods^{11,12}. See Interpretation section for additional details. Characteristic colonies can be subcultured and tested as *Escherichia coli*.

AFNOR Certification has certified 3M Petrifilm CC Plate in comparison to ISO method 4831² and ISO method 4832¹ for enumeration of total coliforms. AFNOR Certification has also certified 3M Petrifilm CC Plate in comparison to NF V08-060³, for enumeration of thermotolerant coliforms.

Safety

The user should read, understand, and follow all safety information in the instructions for the 3M Petrifilm CC Plate. Retain the safety instructions for future reference.

⚠ WARNING: Indicates a hazardous situation, which, if not avoided, could result in death or serious injury and/or property damage

⚠ WARNING

To reduce the risks associated with exposure to biohazards and environmental contamination:

- Follow current industry standards and local regulations for disposal of biohazardous waste.

To reduce the risks associated with release of contaminated product:

- Follow all product storage instruction contained in the instructions for use.
- Do not use beyond the expiration date.

To reduce the risks associated with bacterial infection and workplace contamination:

- Perform 3M Petrifilm CC Plate testing in a properly equipped laboratory under the control of a skilled microbiologist.
- The user must train its personnel in current proper testing techniques: for example, Good Laboratory Practices⁴, ISO 7218⁵, or ISO 17025⁶.

To reduce the risks associated with misinterpretation of results:

- 3M has not documented 3M Petrifilm CC Plates for use in industries other than food and beverage including bottled water. For example, 3M has not documented 3M Petrifilm CC Plates for testing pharmaceuticals, or cosmetics. 3M has not documented 3M Petrifilm CC Plates for testing surface and municipal waters, or waters used in the pharmaceutical or cosmetic industries.
- The use of 3M Petrifilm CC Plates to test water samples in compliance with local water testing regulations is at the sole discretion and responsibility of the end-user. 3M Petrifilm CC Plates have not been tested with all possible bottled water samples, testing protocols or with all possible strains of microorganisms.
- Do not use the 3M Petrifilm CC Plates in the diagnosis of conditions in humans or animals.



- 3M Petrifilm CC Plates do not differentiate any one coliform strain from another.
- Foods with high sugar content may increase the potential for gas production from non-coliform *Enterobacteriaceae*.

Consult the Safety Data Sheet for additional information.

For information on documentation of product performance, visit our website at www.3M.com/foodsafety or contact your local 3M representative or distributor.

User Responsibility

Users are responsible for familiarizing themselves with product instructions and information. Visit our website at www.3M.com/foodsafety, or contact your local 3M representative or distributor for more information.

When selecting a test method, it is important to recognize that external factors such as sampling methods, testing protocols, sample preparation, handling, and laboratory technique may influence results.

It is the user's responsibility in selecting any test method or product to evaluate a sufficient number of samples with the appropriate matrices and microbial challenges to satisfy the user that the chosen test method meets the user's criteria.

It is also the user's responsibility to determine that any test methods and results meet its customers' and suppliers' requirements.

As with any test method, results obtained from use of any 3M Food Safety product do not constitute a guarantee of the quality of the matrices or processes tested.

Limitation of Warranties / Limited Remedy

EXCEPT AS EXPRESSLY STATED IN A LIMITED WARRANTY SECTION OF INDIVIDUAL PRODUCT PACKAGING, 3M DISCLAIMS ALL EXPRESS AND IMPLIED WARRANTIES, INCLUDING BUT NOT LIMITED TO, ANY WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR USE. If any 3M Food Safety Product is defective, 3M or its authorized distributor will, at its option, replace or refund the purchase price of the product. These are your exclusive remedies. You must promptly notify 3M within sixty days of discovery of any suspected defects in a product and return it to 3M. Please call Customer Service (1-800-328-1671 in the U.S.) or your official 3M Food Safety representative for a Returned Goods Authorization.

Limitation of 3M Liability

3M WILL NOT BE LIABLE FOR ANY LOSS OR DAMAGES, WHETHER DIRECT, INDIRECT, SPECIAL, INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING BUT NOT LIMITED TO LOST PROFITS. In no event shall 3M's liability under any legal theory exceed the purchase price of the product alleged to be defective.

Storage

Store unopened 3M Petrifilm CC Plate pouches refrigerated or frozen at temperatures lower than or equal to 8°C (46°F). Just prior to use, allow unopened 3M Petrifilm CC Plate pouches to come to room temperature before opening. Return unused 3M Petrifilm CC Plates to pouch. Seal by folding the end of the pouch over and applying adhesive tape. **To prevent exposure to moisture, do not refrigerate opened pouches.** Store resealed pouches in a cool dry place for no longer than four weeks. It is recommended that resealed pouches of 3M Petrifilm CC Plates be stored in a freezer (see below) if the laboratory temperature exceeds 25°C (77°F) and/or the laboratory is located in a region where the relative humidity exceeds 50% (with the exception of air-conditioned premises).

To store opened pouches in a freezer, place 3M Petrifilm CC Plates in a sealable container. To remove frozen 3M Petrifilm CC Plates for use, open the container, remove the plates that are needed and immediately return remaining plates to the freezer in the sealed container. 3M Petrifilm CC Plates should not be used past their expiration date. The freezer that is used for open pouch storage must not have an automatic defrost cycle as this would repeatedly expose the 3M Petrifilm CC Plates to moisture which can damage the plates.

Do not use 3M Petrifilm CC Plates that show discoloration. Expiration date and lot number are noted on each package of 3M Petrifilm CC Plates. The lot number is also noted on individual 3M Petrifilm CC Plates.

⚠ Disposal

After use, 3M Petrifilm CC Plates may contain microorganisms that may be a potential biohazard.

Follow current industry standards for disposal.

Instructions for Use

Follow all instructions carefully. Failure to do so may lead to inaccurate results.



Preparation, Incubation and Interpretation of Samples from Food and Beverage Industries (Bottled Water Excepted) Including Environmental Samples

Sample Preparation

1. Use appropriate sterile diluents:

Butterfield's phosphate buffered dilution water⁷, 0.1% peptone water⁸, peptone salt diluents^{8,9}, Buffered Peptone Water^{8,9}, saline solution (0.85-0.90%), bisulfite-free letheen broth or distilled water. See section "**Specific Instructions for Validated Methods**" for specific requirements.

Do not use diluents containing citrate, bisulfite or thiosulfate with 3M Petrifilm CC Plates; they can inhibit growth. If citrate buffer is indicated in the standard procedure, substitute with one of the buffers listed above, warmed to 40-45°C (104-113°F).

2. Blend or homogenize sample.
3. For optimal growth and recovery of microorganisms, adjust the pH of the sample suspension to 6.6 - 7.2. For acidic products, adjust the pH with 1N NaOH. For alkaline products, adjust the pH with 1N HCl.

Plating

1. Place the 3M Petrifilm CC Plate on a flat, level surface.
2. Lift the top film and with the pipette perpendicular to the inoculation area dispense 1 mL of sample suspension onto the center of bottom film.
3. Roll the top film down onto the sample to prevent trapping air bubbles.
4. Place the 3MTM PetrifilmTM Spreader with the flat side down on the center of the 3M Petrifilm CC Plate. Press gently on the center of the 3M Petrifilm Spreader to distribute the sample evenly. Spread the inoculum over the entire 3M Petrifilm CC Plate growth area before the gel is formed. Do not slide the 3M Petrifilm Spreader across the film.
5. Remove the 3M Petrifilm Spreader and leave the 3M Petrifilm CC Plate undisturbed for at least one minute to permit the gel to form.

Incubation

Incubate 3M Petrifilm CC Plates in a horizontal position with the clear side up in stacks of no more than 20 plates. Several incubation times and temperatures can be used depending on current local reference methods, some of which are listed in the "**Specific Instructions for Validated Methods**" section.

Interpretation

1. 3M Petrifilm CC Plates can be counted using a standard colony counter or other illuminated magnifier. Gas produced by coliform may disrupt the colony so that the colony "outlines" the bubble. This should be counted as a single coliform. Do not count colonies on the foam dam since they are removed from the selective influence of the medium. Do not count artifact bubbles that may be present.

The interpretation of coliform colonies on the 3M Petrifilm CC Plate varies by reference method. For example:

AOAC® Official MethodsSM:

Coliform colonies are red and closely associated (within one colony diameter) with entrapped gas. Colonies not associated with gas (a distance greater than one colony diameter between colony and gas bubble) are not counted as coliforms.

OR NF Validation certified methods:

- As compared to ISO method 4831² (MPN method), coliform colonies are red and closely associated (within one colony diameter) with entrapped gas.
- As compared to ISO method 4832¹ (total coliforms) and NF V08-060³ (fecal coliforms) both based on VRBL methods, count all red colonies with or without gas.

2. The circular growth area is approximately 20 cm². Estimates can be made on 3M Petrifilm CC Plates containing greater than 150 colonies by counting the number of colonies in one or more representative squares and determining the average number per square. Multiply the average number by 20 to determine the estimated count per 3M Petrifilm CC Plate.
3. When colonies are present in large numbers, 3M Petrifilm CC Plates may have a deepening of the gel color and either or both of the following characteristics: many small, indistinct colonies and/or many gas bubbles. High concentrations of coliforms will cause the growth area to turn dark red. When this occurs, record results as too numerous to count (TNTC). When an actual count is required, plate at a higher dilution.
4. Where necessary, colonies may be isolated for further identification. Lift the top film using proper testing technique and pick the colony from the gel. Test using standard procedures.
5. If the 3M Petrifilm CC Plates cannot be counted within 1 hour of removal from the incubator, they may be stored for later enumeration by freezing in a sealable container at temperatures lower than or equal to negative 15°C (5°F) for no longer than one week.



Preparation, Incubation and Interpretation of Bottled Water Samples

Hydrate 3M Petrifilm CC Plates

1. Place the 3M Petrifilm CC Plate on a flat, level surface.
2. Lift the top film and dispense 1 mL of an appropriate sterile hydration diluent onto the center of bottom film. Appropriate sterile hydration diluents include distilled water, deionized (DI) water and reverse osmosis (RO) water.
3. Roll the top film down onto the sample to prevent trapping air bubbles.
4. Place the 3M Petrifilm Spreader with the flat side down on the center of the plate. Press gently on the center of the spreader to distribute the diluent evenly. Spread the diluent over the entire 3M Petrifilm Plate growth area before the gel is formed. Do not slide the spreader across the film.
5. Remove the spreader and allow the plates to remain closed for a minimum of 1 hour before use.
6. Store hydrated 3M Petrifilm CC Plates in a sealed pouch or plastic bag. Protect plates from light and refrigerate at 2-8°C (36-46°F) for up to 7 days.

Water Filtration and Plate Incubation

1. Following standard procedures for water analysis, membrane filter water sample using a 47 mm, 0.45 micron pore size Mixed Cellulose Ester (MCE) filter.
2. Carefully lift the top film of the 3M Petrifilm CC Plate. Avoid touching the circular growth area. Place the filter in the center of the hydrated area. Minimize trapping bubbles under the filter.
3. Slowly roll top film onto the filter. Minimize trapping air bubbles and creating gaps between the filter and the 3M Petrifilm CC Plate.
4. Lightly apply pressure by using the 3M Petrifilm Plate spreader or sliding a finger lightly across the entire disk area (including edges) to ensure uniform contact of the filter with the gel and to eliminate any air bubbles.
5. Incubate 3M Petrifilm CC Plates at 35°C ± 1°C for 24 hours ± 2 hours¹¹ or 36°C ± 1°C for 24 hours ± 2 hours¹² in a horizontal position with the clear side up in stacks of no more than 20.

Interpretation

1. 3M Petrifilm CC Plates can be counted using a standard colony counter or other illuminated magnifier. Do not count colonies on the foam dam since they are removed from the selective influence of the medium. Do not count artifact bubbles that may be present.
2. Red colonies associated with gas bubbles are counted as coliforms. Gas bubbles may form a circular or star-shaped pattern around the colony. Gas produced by coliforms may disrupt the colony so that the colony “outlines” the bubble. This should be counted as a single coliform. Red colonies without closely associated gas bubbles may be coliforms and should be picked and tested with appropriate confirmation methods^{11,12}.
3. When colonies are present in large numbers, 3M Petrifilm CC Plates will have a deepening of the gel color associated with many small, indistinct colonies or gas bubbles. When this occurs, record results as too numerous to count (TNTC).
4. Colonies may be isolated for further identification. Lift the top film and pick the colony from the gel or the filter surface. When lifting the top film, the filter may adhere to either the top film or the bottom film. If the filter adheres to the top film, separate the filter from the top film and pick colonies. Test using standard procedures.

Note: Delayed counting of 3M Petrifilm CC Plates with filters is not recommended.

For further information refer to the appropriate “3M™ Petrifilm™ Coliform Count Plate Interpretation Guide.” If you have questions about specific applications or procedures, please visit our website at www.3M.com/foodsafety or contact your local 3M representative or distributor.

Specific Instructions for Validated Methods

AOAC® Official MethodsSM (986.33 Bacteria and Coliform Counts in Milk, Dry Rehydratable Film Methods and 989.10 Bacterial and Coliforms Counts in Dairy Products, Dry Rehydratable Film Methods)

Incubate 3M Petrifilm CC Plates 24 hours ± 2 hours at 32°C ± 1°C.

AOAC® Official MethodsSM (991.14 Coliform and Escherichia coli Counts in Foods, Dry Rehydratable Film Methods)

Incubate 3M Petrifilm CC Plates 24 hours ± 2 hours at 35°C ± 1°C.

NF Validation by AFNOR Certification:

NF Validation certified method in compliance with ISO 16140-2¹⁰ in comparison to ISO 4832¹ (3M-01/2-09/89 A)

Scope of the validation: For testing all human food products (except raw shellfish), pet food and environmental samples. Use the following details when implementing the above Instructions for Use:

Sample preparation:

Use only ISO listed diluents.

Incubation:

Incubate 3M Petrifilm CC Plates 24 hours \pm 2 hours at 30°C \pm 1°C or 37°C \pm 1°C.

Interpretation:

Calculate the number of microorganisms present in the test sample according to ISO 7218⁵ for one plate per dilution. Estimates are outside of the scope of the NF Validation certification (cf interpretation part paragraph 2).

NF Validation certified method in compliance with ISO 16140-2¹⁰ in comparison to ISO 4831² (3M-01/2-09/89 B)

Scope of the validation: For testing all human food products (except raw shellfish).

Use the following details when implementing the above Instructions for Use:

Sample preparation:

Use only ISO listed diluents.

Incubation:

Incubate 3M Petrifilm CC Plates 24 hours \pm 2 hours at 30°C \pm 1°C or 37°C \pm 1°C.

Interpretation:

Calculate the number of microorganisms present in the test sample according to ISO 7218⁵ for one plate per dilution. Estimates are outside of the scope of the NF Validation certification (cf interpretation part paragraph 2).

NF Validation certified method in compliance with ISO 16140-2¹⁰ in comparison to NF V08-060³ (3M-01/2-09/89 C)

Scope of the validation: For testing all human food products

Use the following details when implementing the above Instructions for Use:

Sample preparation:

Use only ISO listed diluents

Incubation:

Incubate 3M Petrifilm CC Plates 24 hours \pm 2 hours at 44°C \pm 1°C.

Interpretation:

Calculate the number of microorganisms present in the test sample according to ISO 7218⁵ for one plate per dilution. Estimates are outside of the scope of the NF Validation certification (cf interpretation part paragraph 2).



**3M 01/2 – 09/89A, 01/2 – 09/89B, 01/2 – 09/89C
ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS**

<http://nf-validation.afnor.org/en>

For more information about end of validity, please refer to NF VALIDATION certificate available on the website mentioned above.



References

1. ISO 4832. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coliforms – Colony count technique.
2. ISO 4831. Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of coliforms – Most probable number technique.
3. AFNOR NF V08-060. General guidance for the enumeration of fecal coliforms – Colony count technique (VRBL) at 44°C – Routine method.
4. U.S. Food and Drug Administration. Code of Federal Regulations, Title 21, Part 58. Good Laboratory Practice for Nonclinical Laboratory Studies.
5. ISO 7218. Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations.
6. ISO/IEC 17025. General requirements for the competence of testing and calibration laboratories.
7. FDA. Bacteriological Analytical Manual (BAM), 8th Edition, Revision A, 1998. Reagents Index for BAM found at: <http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm055791.htm>.
8. ISO 6887-5. Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination, Part 5: Specific rules for the preparation of milk and milk products.
9. ISO 6887-1. Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination, Part 1: General rules for the preparation of the initial suspension and decimal dilutions.
10. ISO 16140-2. Microbiology of the food chain – Method validation – Protocol for the validation of alternative (proprietary) methods against a reference method.
11. U.S. Food and Drug Administration. 2002. Bacteriological Analytical Manual, Ch. 4, Section III, Method 4. Membrane filter method for coliforms.
12. American Public Health Association. 2017. Standard Methods for the Examination of Water and Wastewater, 20th Ed. Method 9222B2a.

Refer to the current versions of the standard methods listed above.

Explanation of Symbols

www.3M.com/foodsafety/symbols

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