3M | Petrifilm™

6404/6414/6444

Product Instructions

- (EN) *E. coli* / Coliform Count Plate
- (FR) Test pour la numération des *E.coli* et Coliformes
- (DE) *E. coli* und Coliforme Zählplatte
- (IT) Piastra per il conteggio di *E. coli* e Coliformi
- (ES) Placa para recuento de *E.coli* y Coliformes
- (NL) *E. coli* / Coliform Telplaat
- (SV) Odlingsplatta för *E. coli* och koliforma bakterier
- (DA) *E. coli* / Coliform Tælleplade
- (NO) for *E. coli*- / koliforme bakterier
- (FI) *E. colin* ja koliformien kasvatusalusta
- (PT) Placa para Contagem de *E. coli* / Coliformes
- (EL) Πλακίδιο Καταμέτρησης *E. coli* / Κολοβακτηριδίων
- (PL) Płytka do oznaczania liczby *E. coli* / Coliform
- (RU) Тест-пластина для подсчета *E. coli* и колиформных бактерий
- (TR) *E. coli* / Koliform Sayım Plakası
- (JA) *E.coli*および大腸菌群数測定用プレート（ＥＣプレート）
- (ZH) *大肠杆菌*/大肠菌群测试片
- (TH) แผ่นอาหารเลี้ยงเชื้อสำหรับนับจำนวนเชื้อ*อีโคไล* / โคลิฟอร์ม
- (KO) *대장균* / 대장균군 측정용 플레이트

EC

***E. coli* / Coliform Count**

3M | Petrifilm™

Product Instructions

E. coli / Coliform Count Plate

Product Description and Intended Use

The 3M™ Petrifilm™ *E. coli* / Coliform Count (EC) Plate is a sample-ready-culture medium system which contains modified Violet Red Bile (VRB) nutrients, a cold-water-soluble gelling agent, an indicator of glucuronidase activity, 5-bromo-4-chloro-3-indolyl-D-glucuronide (BCIG), and a tetrazolium indicator that facilitates colony enumeration. 3M Petrifilm EC Plates are used for the enumeration of *Escherichia coli (E. coli)* and coliforms in the food and beverage industries. 3M Petrifilm EC Plate components are decontaminated though not sterilized. 3M Food Safety is certified to International Standards Organization (ISO) 9001 for design and manufacturing. 3M Petrifilm EC Plate has not been evaluated with all possible food products, food processes, testing protocols or with all possible microorganism strains.

Safety

The user should read, understand, and follow all safety information in the instructions for 3M Petrifilm EC 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

Do not use this plate for the detection of *E. coli* O157. Because most *E. coli* O157 strains are atypical, for example they are glucuronidase negative, they will not produce a blue color, and will not be detected on 3M Petrifilm EC Plates.

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 EC Plate testing in a properly equipped laboratory under the control of a skilled microbiologist.
- The user must train personnel in current proper testing techniques: for example, Good Laboratory Practices[1], ISO 17025[2] or ISO 7218[3].

To reduce the risks associated with misinterpretation of results:

- 3M has not documented 3M Petrifilm EC Plates for use in industries other than food and beverage. For example, 3M has not documented 3M Petrifilm EC Plates for testing water, pharmaceuticals, or cosmetics.
- Do not use 3M Petrifilm EC Plates in the diagnosis of conditions in humans or animals.
- The 3M Petrifilm EC Plates do not differentiate any one microorganism strain from another.

Consult the Safety Data Sheet for additional information.

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.

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 EC Plate pouches refrigerated or frozen at temperatures lower than or equal to 8°C (46°F). Just prior to use, allow unopened pouches to come to room temperature before opening. Return unused 3M Petrifilm EC 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 EC 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 EC Plates in a sealable container. To remove frozen 3M Petrifilm EC 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 EC 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 plates to moisture which can damage the plates.

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

⚠ Disposal

After use, 3M Petrifilm EC Plates may contain microorganisms that may be a potential biohazard. Follow current industry standards for disposal.

Instructions for Use

Sample Preparation

1. Use appropriate sterile diluents:

   Butterfield's phosphate buffered dilution water[4], 0.1% peptone water, peptone salt diluent, quarter-strength Ringer's solution, saline solution (0.85-0.90%), bisulfite-free letheen broth or distilled water.

   Do not use diluents containing citrate, bisulfite or thiosulfate with 3M Petrifilm EC 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.
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 1*N* NaOH. For alkaline products, adjust the pH with 1*N* HCl.

Plating

1. Place the 3M Petrifilm EC 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 3M™ Petrifilm™ Spreader with the flat side down on the center of the plate. Press gently on the center of the 3M Petrifilm Spreader to distribute the sample evenly. Spread the inoculum over the entire 3M Petrifilm EC 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 EC Plate undisturbed for at least one minute to permit the gel to form.

Incubation

Incubate 3M Petrifilm EC 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 EC 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.

   The interpretation of *E. coli* colonies on the 3M Petrifilm EC Plate is as follows:

   AOAC Official Methods (998.08 and 991.14) – enumerate blue to red-blue colonies associated with entrapped gas, regardless of size or intensity of color, as confirmed *E. coli*. Blue colonies without gas are not counted as *E. coli*.

   Other 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. The total coliform count consists of both the red and blue colonies associated with gas at 24 hours. Anytime within the validated method incubation period that a blue colony associated with gas appears, it is a confirmed *E. coli*.

⚠ WARNING

Do not use this plate for the detection of *E. coli* O157. Because most *E. coli* O157 strains are atypical, for example they are glucuronidase negative, they will not produce a blue color, and will not be interpreted as *E. coli* on 3M Petrifilm EC Plates.

2. The circular growth area is approximately 20 cm^2. Estimates can be made on 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 plate.
3. When present in large numbers, 3M Petrifilm EC Plates may have one or more of the following characteristics: a deepening of the gel color with many small, indistinct colonies; and many gas bubbles. High concentrations of *E. coli* will cause the growth area to turn blue, while high concentrations of coliforms (non-*E. coli*) 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 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 for no longer than one week.

For further information refer to the "3M™ Petrifilm™ *E. coli*/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 official 3M representative or distributor.

Specific Instructions for Validated Methods

AOAC® Official Methods℠ (998.08 Confirmed *Escherichia coli* Counts in Poultry, Meats and Seafood, Dry Rehydratable Film Method)

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

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

For coliform results incubate 3M Petrifilm EC Plates 24 hours ± 2 hours at 35°C ± 1°C.

For *E. coli* results incubate 3M Petrifilm EC Plates an additional 24 hours ± 2 hours (48 hours ± 4 hours total) at 35°C ± 1°C.

References

1. U.S. Food and Drug Administration. Code of Federal Regulations, Title 21, Part 58. Good Laboratory Practice for Nonclinical Laboratory Studies.
2. ISO/IED 17025. General requirements for the competence of testing and calibration laboratories.
3. ISO 7218. Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations.
4. 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.
5. ISO 6887. Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination.

Explanation of Symbols

www.3M.com/foodsafety/symbols

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