

## Product Instructions

-  (EN) Rapid *E. coli* / Coliform Count Plate
-  (FR) Test Rapide *E. coli* / Coliformes
-  (DE) Rapid *E. coli* / Coliform Zählplatte
-  (IT) Piastra per il conteggio rapido di *E. coli* e Coliformi
-  (ES) Placa para Recuento Rápido de *Escherichia coli* / Coliformes
-  (NL) Snelle *E. coli* / Coliform Telplaat
-  (SV) Rapid *E. coli* / Coliform Count Plate
-  (DA) Rapid *E. coli* / Coliform Tælleplade
-  (NO) Hurtigfilm for *E. coli* / koliforme bakterier
-  (FI) Nopea *E. coli* / koliformien kasvatusalusta
-  (PT) Placa para Contagem rápida de *E. coli* / Coliformes (REC)
-  (EL) Πλακίδιο ταχείας καταμέτρησης *E. coli*/κολοβακτηριδίων
-  (PL) Płytko do szybkiego oznaczania liczby *E. coli* / Coliform Count
-  (RU) Тест-пластина для быстрого подсчета *E. coli*/БГКП
-  (TR) Hızlı *E. coli* / Koliform Sayım Plakası
-  (JA) *E. coli*および大腸菌群数迅速測定用プレート
-  (ZH) 快速大肠杆菌/大肠菌群 (REC) 测试片
-  (TH) Rapid *E. coli* / Coliform Count Plate
-  (KO) 속성 대장균/대장균군용 플레이트

**REC**Rapid *E. coli* / Coliform

## Product Instructions

# Rapid *E. coli* / Coliform Count Plate

### Product Description and Intended Use

The 3M™ Petrifilm™ Rapid *E. coli* / Coliform Count (REC) Plate is a selective and differential sample-ready-culture-medium system which contains proprietary nutrients, a cold-water-soluble gelling agent, 5-bromo-4-chloro-3-indolyl-D-glucuronide (BCIG) an indicator of glucuronidase activity, and a tetrazolium indicator that facilitates colony enumeration. The 3M Petrifilm REC Plate is used for the enumeration of *Escherichia coli* (*E. coli*) and coliforms in the food and beverage industries. The 3M Petrifilm REC Plate components are decontaminated though not sterilized. 3M Food Safety is certified to ISO (International Organization for Standardization) 9001 for design and manufacturing. The 3M Petrifilm REC 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 the 3M Petrifilm REC 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 specific 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 therefore will be detected as coliforms on 3M Petrifilm REC Plates.**

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

- After use, 3M Petrifilm REC Plates may contain microorganisms that may be a potential biohazard. 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 instructions contained in the instructions for use.
- Do not use beyond the use by date.
- Do not use 3M Petrifilm REC Plates that show discoloration.
- Do not use diluents containing citrate, bisulfate or thiosulfate with the 3M Petrifilm REC Plate; they can inhibit growth.

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

- Perform 3M Petrifilm REC 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 Practices<sup>1</sup>, ISO 7218<sup>2</sup>, or ISO 17025<sup>3</sup>.

**To reduce the risks associated with misinterpretation of results:**

- 3M has not documented 3M Petrifilm REC Plates for use in industries other than food and beverage. For example, 3M has not documented 3M Petrifilm REC Plates for testing water, pharmaceuticals, or cosmetics.
- Do not use 3M Petrifilm REC Plates in the diagnosis of conditions in humans or animals.
- 3M Petrifilm REC Plates do not differentiate any one *E. coli* or coliform strain from another.
- A few strains of bacteria can produce  $\beta$ -glucuronidase such as *Shigella*, *Salmonella*, *Enterobacter*, *Citrobacter* and *Klebsiella* and may produce blue to blue-green colonies on the 3M Petrifilm REC Plate.
- 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](http://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](http://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. The food sample itself 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 REC Plate pouches refrigerated or frozen (-20 to 8°C / -4 to 46°F). Just prior to use, allow unopened 3M Petrifilm REC Plate pouches to come to room temperature before opening (20-25°C (68-77°F) / <60% RH). Return unused 3M Petrifilm REC 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 3M Petrifilm REC Plate pouches in a cool dry place for no longer than four weeks. It is recommended that resealed pouches of 3M Petrifilm REC Plates be stored in a freezer for no longer than four weeks 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 the 3M Petrifilm REC Plates in a sealable container. To remove the frozen 3M Petrifilm REC Plates for use, open the container, remove the plates that are needed and immediately return remaining plates to the freezer in the sealed container. The freezer that is used for open pouch storage must not have an automatic defrost cycle as this would repeatedly expose the 3M Petrifilm REC Plates to moisture which can damage the plates.

Do not use 3M Petrifilm REC Plates that show any visible discoloration. Use by date and batch code are noted on each pouch of 3M Petrifilm REC Plates. The batch code is also noted on individual 3M Petrifilm REC Plates. 3M Petrifilm REC Plates should not be used past their use by date.

### **△ Disposal**

After use, 3M Petrifilm REC Plates may contain microorganisms that may be a potential biohazard. Follow current industry standards and local regulations for disposal of biohazardous waste.

### **Instructions for Use**

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

#### **Sample Preparation**

1. Use appropriate sterile diluents:

Butterfield's phosphate-buffered dilution water, 0.1% peptone water, peptone salt diluent (Maximum Recovery Diluent), buffered peptone water, 0.85%-0.9% saline, phosphate buffered saline (PBS), distilled water or bisulfite-free letheen broth.

**Do not use diluents containing citrate, bisulfite or thiosulfate with the 3M Petrifilm REC Plates; they can inhibit growth.** If citrate buffer is indicated in the standard procedure, substitute with Butterfield's phosphate-buffered dilution water, warmed to 40-45°C.

2. Blend or homogenize the sample.
3. For optimal growth and recovery of microorganisms in acidic products (< pH 5), adjust the pH of the sample suspension to greater than pH 5. For acidic products, adjust the pH with 1N NaOH.

## Plating

1. Place the 3M Petrifilm REC 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™ Flat Spreader (6425) with the flat side down on the center of the plate. Press gently on the center of the spreader to distribute the sample evenly. Spread the inoculum over the entire 3M Petrifilm REC Plate growth area before the gel is formed. Do not slide the spreader across the film.
5. Remove the 3M Petrifilm Flat Spreader and leave the plate undisturbed for at least one minute to permit the gel to form.

## Incubation

Incubate the 3M Petrifilm REC 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 the current local reference methods, some of which are listed in the “Specific Instructions for Validated Methods” section.

## Interpretation

1. 3M Petrifilm REC 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. Interpretation of *E. coli* colonies is as follows:  
Enumerate blue to blue-green colonies with and without gas, regardless of size or intensity of color, as *E. coli*.

## WARNING

**Do not use this plate for the specific 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 therefore will be detected as coliforms on 3M Petrifilm REC Plates.**

3. The interpretation of non-*E. coli* coliform colonies on the 3M Petrifilm REC Plate varies by reference method. For example:
  - a. The United States Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM) Chapter 4: Enumeration of *Escherichia coli* and the Coliform Bacteria<sup>10</sup>, defines coliforms as Gram negative rods, which produce acid and gas from lactose during metabolic fermentation. Non-*E. coli* coliform colonies on the 3M Petrifilm REC Plate 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 colonies with gas and blue colonies with and without gas.
  - b. ISO defines coliforms by their ability to grow in method-specific, selective media. ISO 4832<sup>4</sup> enumerates typical coliform colonies on Violet Red Bile Lactose (VRBL) agar, with confirmation of atypical colonies. On the 3M Petrifilm REC Plate, non-*E. coli* coliforms are red colonies with and without gas production. The total coliform count is indicated by red colonies with or without gas production and blue colonies with and without gas production.
4. Enumeration:
  - a. Estimates for *E. coli* can be made on 3M Petrifilm REC Plates containing more than 100 blue to blue green colonies.
  - b. Estimates for total coliform can be made on 3M Petrifilm REC Plates containing more than 100 colonies.
  - c. *E. coli* or total coliform may occur on separate dilutions.

The circular growth area is approximately 30 cm<sup>2</sup>. Count the number of colonies in one or more representative squares and determine the average number per square. Multiply the average number by 30 to determine the estimated count per 3M Petrifilm REC Plate.
5. 3M Petrifilm REC Plates with colony counts too numerous to count (TNTC) may have one or more of the following characteristics: lightening of the gel color to yellow, many small, indistinct red or blue colonies and/or many gas bubbles. High concentrations of *E. coli* or coliforms may cause the outer edge of the growth area to turn pink to pink orange. When this occurs, record results as TNTC. For a more accurate count, further dilution of the sample may be necessary.
6. When necessary, colonies may be isolated for further identification. Lift the top film and pick the colony from the gel. Test using standard procedures.

7. If the 3M Petrifilm REC Plates cannot be counted within the incubation period, 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.

For further information refer to the “3M™ Petrifilm™ Rapid *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](http://www.3M.com/foodsafety) or contact your local 3M representative or distributor.

**Specific Instructions for Validated Methods**

AOAC® *Official Method Analysis* 2018.13

AOAC® *Performance Tested* <sup>SM</sup> Certificate #051801

In AOAC® *Official Methods of Analysis*<sup>SM</sup> and AOAC® *Performance Tested Methods*<sup>SM</sup> studies, the 3M Petrifilm REC Plate method was found to be equivalent to the average log counts of the United States FDA BAM Chapter 4: Enumeration of *Escherichia coli* and the Coliform Bacteria, ISO 4832: Microbiology of food and animal feeding stuffs –Horizontal method for the enumeration of coliforms –Colony count technique, and ISO 16649: Microbiology of food and animal feeding stuffs –Horizontal method for the enumeration of β-glucuronidase-positive *Escherichia coli* –Part 2: colony count technique at 44°C using 5-bromo-4-chloro-3-indolyl-β-D-glucuronide.

**Scope of the Validation:** Broad range of foods and select environmental surfaces.

**Incubation:**

**Dairy products:**

Incubate the 3M Petrifilm REC Plates 18-24 hours at 30 ± 1°C or 32 ± 1°C for coliforms and *E. coli* or 42 ± 1°C for *E. coli*.

**All other foods:**

Incubate the 3M Petrifilm REC Plates 18-24 hours at 35 ± 1°C or 37 ± 1°C for coliforms and *E. coli* or 42 ± 1°C for *E. coli*.



**MicroVal Certification**

MicroVal certificate number 2017LR76 in compliance with ISO 16140-2:2016<sup>11</sup> in comparison to ISO 4832:2006<sup>4</sup> and ISO 16649-2:2001<sup>9</sup>

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

**Scope of the validation:**

Broad range of foods and select environmental surfaces.

**Incubation:**

**Dairy products:**

Incubate the 3M Petrifilm REC Plates 18-24 hours at 30 ± 1°C for coliforms and *E. coli* or 42 ± 1°C for *E. coli*.

**All other foods:**

Incubate the 3M Petrifilm REC Plates 18-24 hours at 37 ± 1°C for coliforms and *E. coli* or 42 ± 1°C for *E. coli*.

**Interpretation**

Calculate the number of microorganisms present in the test sample according to ISO 7218<sup>2</sup> for one plate per dilution. See Interpretation section, steps 2 through 4.

## 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 7218. Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations.
3. ISO/IEC 17025. General requirements for the competence of testing and calibration laboratories.
4. ISO 4832. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coliforms – Colony count technique.
5. ISO 4831. Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of coliforms – Most probable number technique.
6. NF V08-060. General guidance for the enumeration of fecal coliforms – Colony count technique (VRBL) at 44°C – Routine method.
7. FDA. Bacteriological Analytical Manual (BAM), Reagents Index for BAM found at: <http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm055791.htm>.
8. ISO 6887-1. Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination.
9. ISO 16649-2. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of  $\beta$ -glucuronidase-positive *Escherichia coli* – Part 2: colony count technique at 44°C using 5-bromo-4-chloro-3-indolyl- $\beta$ -D-glucuronide.
10. FDA. Bacteriological Analytical Manual (BAM), 8<sup>th</sup> Edition, Chapter 4: Enumeration of *Escherichia coli* and the Coliform Bacteria online.
11. ISO 16140-2. Microbiology of the food chain – Method Validation – Protocol for the validation of alternative (proprietary) methods against a reference method.

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

## Explanation of Symbols

[www.3M.com/foodsafety/symbols](http://www.3M.com/foodsafety/symbols)

AOAC is a registered trademark of AOAC INTERNATIONAL

*Official Method* is a service mark of AOAC INTERNATIONAL

*Performance Tested Method* is a service mark of AOAC INTERNATIONAL