3M | Petrifilm™

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# Product Instructions

- EN Select *E. coli* Count Plate
- FR Test pour la numération sélective des *E. coli*
- DE selektive *E. coli* (SEC) Zählplatte
- IT Piastra per il conteggio selettivo di *E. coli*
- ES Placa para recuento selectivo de *E. coli*
- NL Select *E. coli* Telplaat
- SV Selektiv odlingsplatta för *E. coli*
- DA Select *E. coli* Tælleplade
- NO Select *E. coli*
- FI Selektiivinen *E. coli* kasvatusalusta
- PT Selecione a Placa para Contagem de *E. coli*
- EL Πλακίδιο Επιλεκτικής Καταμέτρησης *E. coli*
- PL Płytka do selektywnego oznaczania liczby *E. coli*
- RU Тест-пластина для подсчёта *E. coli*
- TR Hızlı *E. coli* Sayım Plakası
- JA *大腸菌*選択用プレート(SECプレート)
- ZH 选择性*大肠杆菌*测试片
- TH แผ่นอาหารเลี้ยงเชื้อสำหรับนับจำนวน*อีโคไล*แบบจำเพาะ
- KO 선택*대장균* 측정용 플레이트

**SEC**
**Select *E. coli***

3M | Petrifilm™

Product Instructions

# Select *E. coli* Count Plate

## Product Description And Intended Use

The 3M™ Petrifilm™ Select *E. coli* Count (SEC) Plate is a sample-ready-culture medium system which contains selective agents, nutrients, a cold-water-soluble gelling agent, and an indicator of glucuronidase activity, 5-bromo-4-chloro-3-indolyl-D-glucuronide (BCIG), which facilitates colony enumeration. 3M Petrifilm SEC Plates are used for the enumeration of *Escherichia coli (E. coli)* in the food and beverage industries. Most *E. coli* strains are thermotolerant and produce beta-glucuronidase, an enzyme that reacts with BCIG indicator in 3M Petrifilm SEC Plates to produce dark green to blue-green colonies. 3M Petrifilm SEC 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 SEC Plates have not been tested with all possible food products, food processes, testing protocols or with all possible strains of *E. coli* or other bacteria.

## Safety

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

⚠ **CAUTION:** Indicates a hazardous situation, which, if not avoided, could result in minor or moderate injury and/or property damage.

### ⚠ WARNING

**Do not use 3M Petrifilm SEC Plate for the detection of *E. coli* O157. Like most other *E. coli* media, the 3M Petrifilm SEC Plate will not specifically indicate whether any O157 strain is present. Because most O157 strains are atypical *E. coli*, for example they are glucuronidase negative and they will not produce a blue-green color, they will not be detected on 3M Petrifilm SEC Plate.**

**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 expiration date.

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

- Perform 3M Petrifilm SEC 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[1], ISO 17025[2], or ISO 7218[3].

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

- 3M has not documented 3M Petrifilm SEC Plates for use in industries other than food and beverage. For example, 3M has not documented 3M Petrifilm SEC Plates for testing water, pharmaceuticals, or cosmetics.
- Do not use 3M Petrifilm SEC Plates in the diagnosis of conditions in humans or animals.
- 3M Petrifilm SEC Plates do not differentiate any one *E. coli* strain from another.
- High sugar content foods may decrease the color reaction of the glucuronidase and produce pale green to colorless colonies.

**To reduce the risks associated with environmental contamination:**

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

### ⚠ CAUTION

A few strains of bacteria other than *E. coli*, such as some *Shigella*, *Salmonella*, *Enterobacter* and *Klebsiella*, can produce beta-glucuronidase and will produce dark green to blue-green colonies on the 3M Petrifilm SEC Plate, especially when incubated at lower than recommended temperatures[4].

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 SEC 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 SEC 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 SEC 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 SEC Plates in a sealable container. To remove frozen 3M Petrifilm SEC 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 SEC 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 SEC Plates to moisture which can damage the plates.

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

## ⚠Disposal

After use, 3M Petrifilm SEC 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.

**Sample Preparation**

1. Use appropriate sterile diluents: Butterfield's phosphate buffered dilution water[5], 0.1% peptone water[5], peptone salt diluent[6], buffered peptone water[6], quarter-strength Ringer's solution[6], dipotassium hydrogen phosphate[6], 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 SEC 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 6.5 - 7.5. 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 SEC 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 3M Petrifilm SEC Plate. Press gently on the center of the 3M Petrifilm Spreader to distribute the sample evenly. Spread the inoculum over the entire 3M Petrifilm SEC 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 SEC Plate undisturbed for at least one minute to permit the gel to form.

**Incubation**

Incubate 3M Petrifilm SEC Plates in a horizontal position with the clear side up in stacks of no more than 20 plates. Incubate 3M Petrifilm SEC Plates 24 hours ± 2 hours at 42°C ± 1°C. Several incubation times and temperatures can be used depending on current local reference methods, some of which are listed in the section below titled "**Specific Instructions for Validated Methods**".

**Interpretation**

1. 3M Petrifilm SEC 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. *E. coli* produce dark green to light-green colonies. Colonies may have gas bubbles associated with them. Count all green to blue-green colonies as *E. coli* whether there are gas bubbles present or not.

**⚠ WARNING**

**Do not use the 3M Petrifilm SEC 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-green color, and will not be interpreted as *E. coli* on the 3M Petrifilm SEC Plates.**

2. The circular growth area is approximately 20 $cm^2$. Estimates can be made on 3M Petrifilm SEC Plates containing greater than 150 colonies by counting the number of colonies in two 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 SEC Plate.
3. When colonies are present in large numbers, 3M Petrifilm SEC Plates will cause the entire growth area to become gray or blue and either or both of the following characteristics: many small, indistinct colonies and/or many gas bubbles. 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 SEC 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.

For further information refer to the "3M™ Petrifilm™ Select *E. coli* 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**
**NF Validation by AFNOR Certification:**
NF Validation certified method in compliance with ISO 16140[7] in comparison to ISO 16649-2[8].

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

**Scope of the validation:**
For testing all human food products, pet food and industrial environmental samples.

**Sample preparation:**
Use only ISO listed diluents.

**Incubation:**
Incubate 3M Petrifilm SEC Plates 24 hours ± 2 hours at 42°C ± 1°C.

**Interpretation:**
Calculate the number of microorganisms present in the test sample according to ISO 7218[3] for one plate per dilution. For calculation, take into account only 3M Petrifilm SEC Plates that contain up to 150 colonies. Estimates are outside of the scope of the NF Validation Certification (cf. interpretation part paragraph 3).

**3M 01/08 –06/01**
**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. U.S. Food and Drug Administration. Code of Federal Regulations, Title 21, Part 58. Good Laboratory Practice for Nonclinical Laboratory Studies.
2. ISO/IEC 17025. General requirements for the competence of testing and calibration laboratories.
3. ISO 7218. Microbiology of food and animal feeding stuffs – General rules for microbiological examination.
4. Frampton, E.W. and L. Restaino, 1993. Methods for *Escherichia coli* identification in food, water and clinical samples based on beta-glucuronidase detection. *Journal of Applied Bacteriology* 74, 223-233.
5. 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.
6. ISO 6887-1 Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination.
7. ISO 16140. Microbiology of food and animals feeding stuffs – Protocol for the validation of alternative methods.
8. ISO 16649-2: Microbiology of food and animal feeding stuffs- Horizontal method for the enumeration of ß-glucuronidase-positive *Escherichia coli* - Part 2: Colony- count technique at 44°C using 5-bromo-4-chloro-3-indolyl ßD-glucuronide.


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

## Explanation Of Symbols

www.3M.com/foodsafety/symbols

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