

Product Instructions

-  (EN) *Enterobacteriaceae* Count Plate
-  (FR) Test *Enterobacteriaceae*
-  (DE) *Enterobacteriaceae* Zählplatte
-  (IT) Piastra per il conteggio di *Enterobacteriaceae*
-  (ES) Placa para recuento de *enterobacterias*
-  (NL) *Enterobacteriaceae* Telplaat
-  (SV) *Enterobacteriaceae* Count Plate
-  (DA) *Enterobacteriaceae* Tælleplade
-  (NO) For *Enterobacteriaceae*
-  (FI) *Enterobakteerien* Kasvatusalusta
-  (PT) Placa para Contagem de *Enterobacteriaceae*
-  (EL) Πλακίδιο Καταμέτρησης *Εντεροβακτηριδίων*
-  (PL) Płytko do oznaczania liczby bakterii z rodziny *Enterobacteriaceae*
-  (RU) Тест-пластина для подсчета *энтеробактерий*
-  (TR) *Enterobacteriaceae* Sayım Plakası
-  (JA) 腸内細菌科菌群数測定用プレート
-  (ZH) 肠杆菌科测试片
-  (TH) *Enterobacteriaceae* Count Plate
-  (KO) 장내세균 측정용 플레이트



Product Instructions

Enterobacteriaceae Count Plate

Product Description and Intended Use

The 3M™ Petrifilm™ *Enterobacteriaceae* Count (EB) Plate is a sample-ready-culture medium system which contains modified Violet Red Bile Glucose (VRBG) nutrients, a cold-water-soluble gelling agent, and a tetrazolium indicator that facilitates colony enumeration. 3M Petrifilm EB Plates are used for the enumeration of *Enterobacteriaceae* in the food, beverage, and bottled water industries. *Enterobacteriaceae* are oxidase-negative, Gram-negative rods that ferment glucose to produce acid and/or gas. On 3M Petrifilm EB Plates, *Enterobacteriaceae* will appear as red colonies with yellow zones, red colonies with gas bubbles, or red colonies with yellow zones and gas bubbles. 3M Petrifilm EB 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 EB Plates have not been evaluated with all possible food products, food processes, testing protocols or with all possible strains for *Enterobacteriaceae* or other bacteria.

Safety

The user should read, understand, and follow all safety information in the instructions for the 3M Petrifilm EB 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 use by date.

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

- Perform 3M Petrifilm EB 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 Practices¹, ISO/EIC 17025² or ISO 7218³.

To reduce the risks associated with misinterpretation of results:

- 3M has not documented 3M Petrifilm EB Plates for use in industries other than food and beverages including bottled water. For example, 3M has not documented 3M Petrifilm EB Plates for testing pharmaceuticals, or cosmetics. 3M has not documented 3M Petrifilm EB Plates for testing surface and municipal waters, or waters used in the pharmaceutical or cosmetic industries.
- The use of 3M Petrifilm EB 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 EB Plates have not been tested with all possible bottled water samples, testing protocols or with all possible strains of microorganisms.
- Do not use 3M Petrifilm EB Plates in the diagnosis of conditions in humans and animals
- 3M Petrifilm EB Plates do not differentiate any one *Enterobacteriaceae* 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 EB Plate pouches refrigerated or frozen at temperatures lower than or equal to 8°C (46°F). Just prior to use, allow unopened 3M Petrifilm EB Plate pouches to come to room temperature before opening. Return unused 3M Petrifilm EB 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 EB Plate pouches in a cool, dry place for no longer than four weeks. It is recommended that resealed pouches of 3M Petrifilm EB 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 EB Plates in a sealable container. To remove frozen 3M Petrifilm EB Plates for use, open the container, remove the plates that are needed and immediately return remaining plates to the freezer in the sealed container. 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 EB Plates that show discoloration. Use by date and batch number are noted on each package of 3M Petrifilm EB Plates. The lot number is also noted on individual 3M Petrifilm EB Plates. The 3M Petrifilm EB Plates should not be used past their use by date.

⚠ Disposal

After use, 3M Petrifilm EB 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 and Incubation 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 water⁴, peptone salt diluent⁵, 0.1% peptone water⁴, buffered peptone water⁵, dipotassium hydrogen phosphate solution⁵, saline solution (0.85 – 0.90%), bisulfate-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 EB 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 EB 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 EB Plate. Press gently on the center of the 3M Petrifilm Spreader to distribute the sample evenly. Spread the inoculum over the entire 3M Petrifilm EB 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 EB Plate undisturbed for at least one minute to permit the gel to form.

Incubation

Incubate 3M Petrifilm EB Plates in a horizontal position with the clear side up in stacks of no more than 20 - 3M Petrifilm EB Plates. Incubate 3M Petrifilm EB Plates 24 hours \pm 2 hours. 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**”.

Preparation and Incubation of Bottled Water Samples

3M Petrifilm EB Plate Hydration

1. Place the 3M Petrifilm EB 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 3M Petrifilm Spreader and allow the plates to remain closed for a minimum of 1 hour before use.
6. Store hydrated 3M Petrifilm EB 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 EB Plate. Avoid touching the circular growth area. Place the filter in the center of the hydrated area.
3. Slowly replace top film onto the membrane filter. Minimize trapping air bubbles and creating gaps between the filter and the 3M Petrifilm EB Plate.
4. Lightly apply pressure by using the 3M Petrifilm 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.

Incubation

Incubate 3M Petrifilm EB Plates at 34°C to 37°C for 24 hours \pm 2 hours in a horizontal position with the clear side up in stacks of no more than 20.

Interpretation

1. 3M Petrifilm EB 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. *Enterobacteriaceae* are red colonies with yellow zones and/or red colonies with gas bubbles with or without yellow zones. Colonies not associated with gas (a distance greater than on colony diameter between colony and gas bubble) and not associated with a yellow zone are not counted as *Enterobacteriaceae*.



3. The circular growth area is approximately 20cm². Estimates can be made on 3M Petrifilm EB Plates containing greater than 100 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 plate.
4. When colonies are present in large numbers, 3M Petrifilm EB Plates will have a deepening of the gel color or the plate may turn completely yellow, 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.
5. 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.
6. If the 3M Petrifilm EB 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.

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

For further information refer to the “3M™ Petrifilm™ *Enterobacteriaceae* 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

Scope of the validation: 2003.01 Enumeration of *Enterobacteriaceae* in Selected Foods

Incubation:

Incubate 3M Petrifilm EB Plates 24 hours ± 2 hours at 37°C ± 1°C.

NF Validation by AFNOR Certification

NF Validation certified method in compliance with ISO 16140-2⁶ in comparison to ISO 21528-2⁷

(3M – 01/6 – 09/97)

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

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

Sample preparation: Use only ISO listed dilutents⁵.

Incubation:

Incubate 3M Petrifilm EB Plates 24 hours ± 2 hours at 30°C ± 1°C or 37°C ± 1°C.

Interpretation:

Calculate the number of microorganisms present in the test sample according to ISO 7218³ for one plate per dilution. For calculation, take into account only 3M Petrifilm EB Plates that contain up to 100 colonies. Estimates are outside of the scope of the NF Validation Certification (cf. interpretation part paragraph 3).



3M 01/06 – 09/97

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.

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