3M | Petrifilm™

Product Instructions

Aerobic Count Plate

Product Description and Intended Use

The 3M™ Petrifilm™ Aerobic Count (AC) Plate is a sample-ready-culture medium system which contains modified Standard Methods nutrients, a cold-water-soluble gelling agent, and a tetrazolium indicator that facilitates colony enumeration. 3M Petrifilm AC Plates are used for the enumeration of aerobic bacteria in the food and beverage industries. 3M Petrifilm AC 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 AC Plates have 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 AC 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 AC 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 AC Plates for use in industries other than food and beverage. For example, 3M has not documented 3M Petrifilm AC Plates for testing water, pharmaceuticals, or cosmetics
- Do not use 3M Petrifilm AC Plates in the diagnosis of conditions in humans or animals.
- Do not use 3M Petrifilm AC Plates for U.S.-recognized laboratory pasteurized counts.
- Acceptance of the 3M Petrifilm AC Plate method for the testing of water per an accepted local government regulation is at the sole discretion and responsibility of the end-user.
- 3M Petrifilm AC Plates do not differentiate any one microorganism strain from another.
- Some strains (such as lactic acid bacteria or some micrococci) may not be detected on 3M Petrifilm AC Plates while some bacterial strains may recover at higher levels compared to plate count agar.

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.

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.

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.

Storage

Store unopened 3M Petrifilm AC 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 (20-25°C / <60% RH). Return unused 3M Petrifilm AC 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 AC 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 AC Plates in a sealable container. To remove frozen 3M Petrifilm AC 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 AC 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 AC Plates that show discoloration. Expiration date and lot number are noted on each package of 3M Petrifilm AC Plates. The lot number is also noted on individual 3M Petrifilm AC Plates.

⚠ Disposal

After use, 3M Petrifilm AC 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:

   Butterfields phosphate buffered dilution water[4], 0.1% peptone water[4], peptone salt diluent[5], buffered peptone water[5], dipotassium hydrogen phosphate solution[5], 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 AC 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 1*N* NaOH. For alkaline products, adjust the pH with 1*N* HCl.

Plating

1. Place the 3M Petrifilm AC Plate on a flat, level surface.
2. Lift the top film and with the pipette perpendicular to the inoculation surface dispense 1 mL of sample suspension onto the center of bottom film.
3. Drop the top film down onto the sample.
4. Place the 3M™ Petrifilm™ Spreader with the recessed side down on the center of the 3M Petrifilm AC Plate. Press gently on the center of the 3M Petrifilm Spreader to distribute the sample evenly. Spread the inoculum over the entire 3M Petrifilm AC 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 AC plate undisturbed for at least one minute to permit the gel to form.

Incubation

Incubate 3M Petrifilm AC Plates in a horizontal position with the clear side up in stacks of no more than 20. 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 AC Plates can be counted using a standard colony counter or other illuminated magnifier. Count all red colonies regardless of size or intensity.
2. The circular growth area is approximately 20 cm^2. Estimates can be made on 3M Petrifilm AC Plates containing greater than 300 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.
3. High concentrations of colonies on the 3M Petrifilm AC Plates will cause the entire growth area to become red or pink. Occasionally, on overcrowded 3M Petrifilm AC Plates, the center may lack visible colonies, but many small colonies can be seen on the edges. When any of these occurs, record results as too numerous to count (TNTC). When an actual count is required, plate at a higher dilution.
4. Some organisms can liquefy the gel, allowing them to spread out and obscure the presence of other colonies. If liquefied gel interferes with counting, an estimated count should be made by counting the unaffected areas.
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 AC 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 less than or equal to negative 15°C (5°F) for no longer than one week.

For further information refer to the "3M™ Petrifilm™ Aerobic 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 Methods℠ (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)

Scope of the validations: Milk and other dairy products.

Incubate 3M Petrifilm AC Plates 48 hours ± 3 hours at 32°C ± 1°C.

Follow guidelines provided in Interpretation section of this document.

AOAC® Official Methods℠ (990.12 Aerobic Plate Count in Foods, Dry Rehydratable Film Methods)

Scope of the validations: In foods.

Incubate 3M Petrifilm AC Plates 48 hours ± 3 hours at 35°C ± 1°C.

Follow guidelines provided in Interpretation section of this document.

NF Validation by AFNOR Certification

NF Validation certified method in compliance with ISO 16140-2[6] in comparison to ISO 4833[7] (3M 01/1-09/89)

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

Scope of the validation:

All human food products, pet food and industrial environmental samples.

Sample Preparation:

Use only ISO listed diluents[5].

Incubation:

Option 1 - For testing all human food products, (**including** dairy products and raw shellfish), pet food and industrial environmental samples:

Incubate 3M Petrifilm AC Plates 72 hours ± 3 hours at 30°C ± 1°C.

Option 2 - For testing all human food products, (**except** dairy products and raw shellfish), pet food and industrial environmental samples:

Incubate 3M Petrifilm AC Plates 48 hours ± 3 hours at 30°C ± 1°C.

Interpretation

Calculate the number of microorganisms present in the test sample according to ISO 7218[3] for one 3M Petrifilm AC Plate per dilution. Estimates are outside of the scope of the NF Validation certification

(See Interpretation section, steps 2 and 4).

3M 01/01-09/89
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 requirements and guidance for microbiological examinations.
4. FDA. Bacteriological Analytical Manual (BAM), 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.
6. ISO 16140-2. Microbiology of the food chain – Method Validation – Protocol for the validation of alternative (proprietary) methods against a reference method.
7. ISO 4833 Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of microorganisms – Colony-count technique at 30°C.

Explanation of Symbols

www.3M.com/foodsafety/symbols