3M | Petrifilm™

6475/6477

Product Instructions

EN Rapid Yeast and Mold Count Plate

RYM
Rapid Yeast and Mold

RAPID

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Product Instructions

Rapid Yeast and Mold Count Plate

Product Description and Intended Use

The 3M™ Petrifilm™ Rapid Yeast and Mold Count (RYM) Plate is a sample-ready-culture-medium system which contains nutrients supplemented with antibiotics, a cold-water-soluble gelling agent, and an indicator system that facilitates yeast and mold enumeration. 3M Petrifilm RYM Plates are used for the enumeration of yeast and mold in the food and beverage industries. 3M Petrifilm RYM Plate components are decontaminated though not sterilized. 3M Food Safety is certified to ISO (International Organization for Standardization) 9001 for design and manufacturing. 3M Petrifilm RYM 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 Product Instructions for the 3M Petrifilm RYM 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 the release of contaminated product:

- Follow all product storage instructions contained in the instructions for use.
- Do not use beyond the expiration date.

To reduce the risks associated with infection and workplace contamination:

- Perform 3M Petrifilm RYM testing in a properly equipped laboratory under the control of a skilled microbiologist.
- The user must train its personnel in proper testing techniques. For example, Good Laboratory Practices[1], ISO 7218[2], or ISO 17025[3].

To reduce the risks associated with misinterpretation of results:

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

Consult the Safety Data Sheet for additional information.

For information on documentation of product performance, 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 RYM Plate pouches refrigerated or frozen (-20 to 8°C / -4 to 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 RYM 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 (20-25°C / <60% RH) for no longer than 4 weeks. It is recommended that resealed pouches of 3M Petrifilm RYM 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 60% (with the exception of air-conditioned premises).

To store opened pouches in a freezer, place 3M Petrifilm RYM Plates in a sealable container. To remove frozen 3M Petrifilm RYM Plates for use, open the container, remove the plates that are needed and immediately return remaining plates to the freezer in the sealed container. Allow 3M Petrifilm RYM Plates to come to room temperature before plating. 3M Petrifilm RYM Plates should not be used past their expiration date. Do not store open pouches in a freezer with an automatic defrost cycle, as this could damage the 3M Petrifilm RYM Plates due to repeated exposure to moisture.

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

⚠ Disposal

After use, 3M Petrifilm RYM Plates may contain microorganisms that may be a potential biohazard. Follow current industry standards for disposal.
For information on potential biohazards, reference Biosafety in Microbiological and Biomedical Laboratories, 6th edition, Section VIII-B: Fungal Agents or equivalent.

Instructions for Use

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

Wear appropriate protective apparel and follow standard good laboratory safety practices (GLP).[1]

Sample Preparation

1. Prepare appropriate dilution(s) of the sample as needed.

   Use appropriate sterile diluents:

   Butterfield's phosphate buffered dilution water, Buffered Peptone Water (ISO), 0.1% peptone water, peptone salt diluent, 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 RYM Plates; they can inhibit growth.** If citrate buffer is indicated in the standard procedure, substitute with 0.1% peptone water, warmed to 40-45°C.

   See "Specific Instructions for Validated Methods" for specific requirements.

2. Blend or homogenize sample.

Plating

1. Place the 3M Petrifilm RYM Plate on a flat, level surface.
2. Lift the top film and with the pipette perpendicular dispense 1 mL of sample suspension onto the center of bottom film.
3. Roll the top film down onto the sample.

4. Place the 3M™ Petrifilm™ Flat Spreader (6425) or other flat spreader on the center of the 3M Petrifilm RYM Plate. Press gently on the center of the spreader to distribute the sample evenly. Spread the inoculum over the entire 3M Petrifilm RYM 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 3M Petrifilm RYM Plate undisturbed for at least one minute to permit the gel to form.

Incubation

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 RYM Plates can be counted using a standard colony counter or other illuminated magnifier. Gridlines are visible with the use of a backlight to assist with estimated enumeration.
2. Do not count colonies on the foam dam since they are removed from the nutrient medium.
3. To differentiate yeast and mold colonies on the 3M Petrifilm RYM Plate, look for one or more of the following characteristics:

YEAST	MOLD
Small colonies	Large colonies
Colonies have defined edges	Colonies have diffuse edges
Pink/tan or blue/green in color	Blue/green to variable upon prolonged incubation
Colonies appear raised (3 dimensional)	Colonies appear flat
Colonies have a uniform color	Colonies have a dark center with diffused edge

4. Read yeast and mold results between 48 to 72 hours depending on the validated method. Certain slower growing yeasts and molds may appear faint at 48 hours. To enhance interpretation of these molds allow for an additional 12 hours of incubation time. If a 60 hour time-point for interpretation is not convenient, extending the incubation time to 72 hours is an acceptable alternative.
5. The circular growth area is approximately 30 cm^2. 3M Petrifilm RYM Plates containing greater than 150 colonies can either be estimated or recorded as Too Numerous To Count (TNTC). Estimation can be done by counting the number of colonies in one or more representative squares and determining the average number per square. The average number can be multiplied by 30 to determine the estimated count per plate. If a more accurate count is required, the sample will need to be retested at higher dilutions. When the sample contains substantial amounts of mold, depending on the type of mold, the upper countable limit may be lowered at user discretion.
6. Food samples may occasionally show interference on the 3M Petrifilm RYM Plates, for example:
   a) a uniform blue background color (often seen from the organisms used in cultured products) these should not be counted as TNTC.
   b) intense, pinpoint blue specs (often seen with spices or granulated products).
7. When necessary, colonies may be isolated for further identification. Lift the top film and pick the colony from the gel.

Specific Instructions for Validated Methods

AOAC® Official Method of Analysis℠ (OMA) #2014.05

AOAC® Research Institute (RI) Performance Tested Method℠ (PTM) #121301

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 121301

In AOAC OMA and PTM studies, the 3M Petrifilm RYM Plate method was found to be equivalent to ISO 21527:2008 parts 1 and 2, and FDA BAM Chapter 18 reference methods at 48 and 60 hours and comparable to Dichloran Rose Bengal Chloramphenicol agar per AOAC SMPR 2021.009 at 60 and 72 hours.

Scope of Validation:

Yogurt, frozen bread dough, fermented salami, sour cream, ready-made pie, frozen ground beef patties, almonds, sandwiches, sliced apples, dehydrated soup and environmental samples from stainless steel, rubber and sealed concreate surfaces. See AOAC OMA 2014.05 and AOAC PTM 121301 for additional validated matrices.

Food samples and environmental samples:

Incubation:

Incubate 3M Petrifilm RYM Plates between 48 and 60 hours at 25°C ± 1 °C or 28°C ± 1°C.

Other validated matrices:

Incubate 3M Petrifilm RYM Plates 60 to 72 hours at 25°C ± 1 °C or 28°C ± 1°C.

Interpretation:

Plates containing greater than 150 colonies can either be estimated or recorded at too numerous to count (TNTC). Estimation can be done by counting the number of colonies in one or more representative squares and determining the average number per square. The average number can be multiplied by 30 to determine the estimated count per plate. If a more accurate count is required, the sample can be retested at higher dilutions.

NF VALIDATION by AFNOR Certification:

NF VALIDATION certified method in compliance with ISO 16140-2[4] in comparison to 21527 part 1 and part 2[5]

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

Scope of the validation:

All human food products, animal feed and industrial production environmental samples
(primary production samples excepted)

Sample preparation:

Use only ISO listed diluents[6]

For beverages, undiluted samples should not be plated.

Incubation:

Incubate 3M Petrifilm RYM Plates between 60 and 72 hours at 25°C ± 1 °C or 28°C ± 1°C.

The plates can be stored in the incubator up to 5 days.

Interpretation:

Count all colonies regardless of color. The separated enumeration of yeasts and molds is outside the scope of the NF VALIDATION Certification. Calculate the number of microorganisms present in the test sample according to ISO 7218[2] for one plate per dilution. For calculation, take into account only 3M Petrifilm RYM Plates that contain up to 150 colonies. Estimates are outside of the scope of the NF Validation Certification (cf. interpretation part paragraph 5). Refer to EN ISO 7218 standard for inoculation, colony counting and calculation and expression of results.

3M 01/13 – 07/14
ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS
www.afnor-validation.com

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 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 16140-2, Microbiology of the food chain – Method Validation – Protocol for the validation of alternative (proprietary) methods against a reference method.
5. ISO 21527. Microbiology of food and animal feeding stuffs- Horizontal method for the enumeration of yeasts and moulds.

   Part 1: Colony count technique in products with water activity greater than 0.95

   Part 2: Colony count technique in products with water activity less than or equal to 0.95
6. ISO 6887. Microbiology of food and animal feeding stuffs- Preparation of test samples, initial suspension and decimal dilutions for microbiological examination.
7. 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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