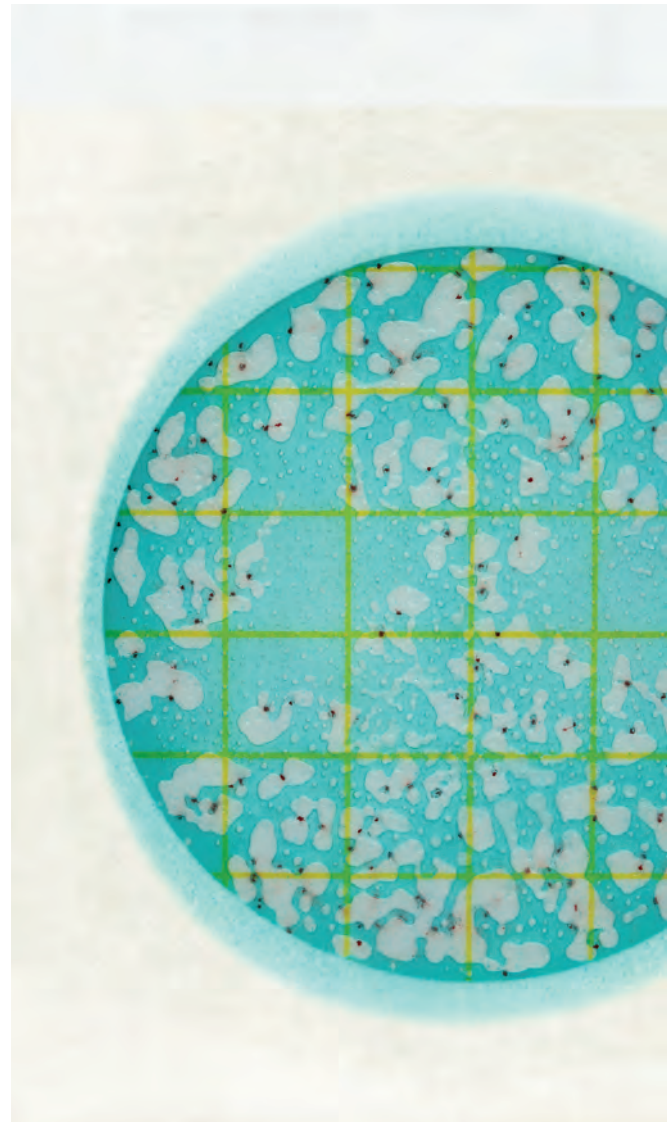




# Petrifilm®

## Interpretation Guide

The Neogen® Petrifilm® Lactic Acid Bacteria Count Plate is a self-contained, sample-ready culture medium system which contains nutrients, selective agents, a cold-water-soluble gelling agent and a tetrazolium indicator that facilitates colony enumeration. The plate contains oxygen scavenging compounds which create an anaerobic environment for the recovery of homofermentative and heterofermentative lactic acid bacteria in the food and beverage industries.



# LAB

Lactic Acid Bacteria Count Plate

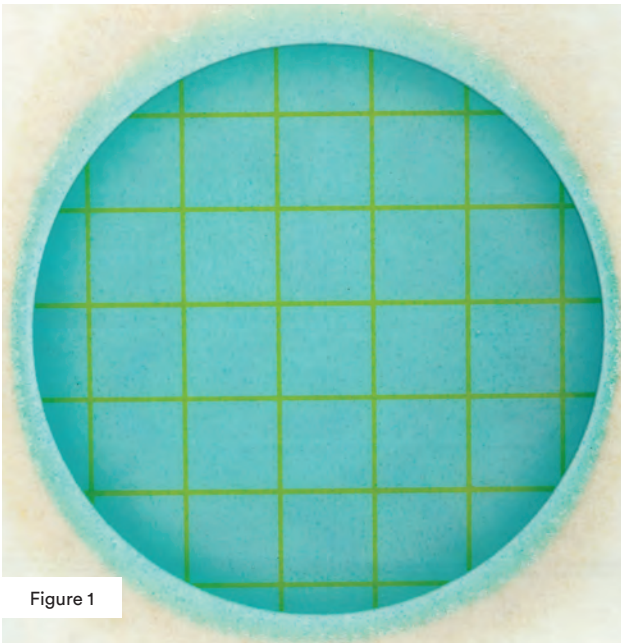


Figure 1

**Total lactic acid bacteria count = 0**

Petrifilm Lactic Acid Bacteria Count Plate without colonies.

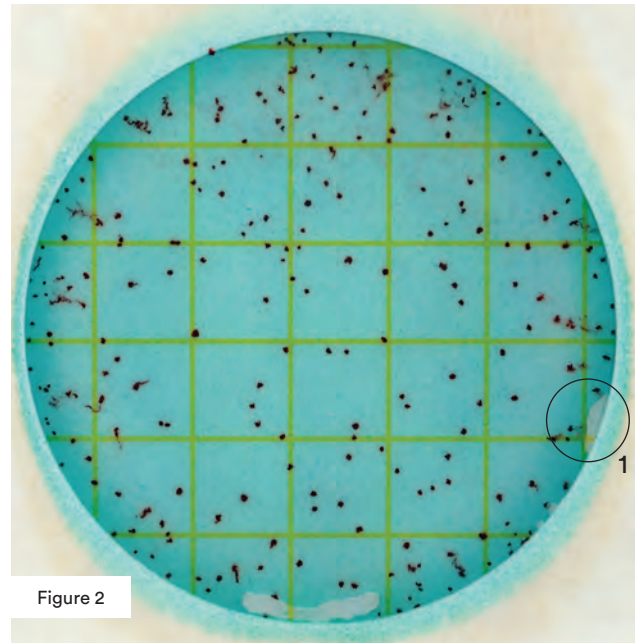


Figure 2

**Total lactic acid bacteria count = 236**

The recommended counting range is less than 300 colonies when colonies without gas are present. Artifact bubbles may result from improper inoculation (Circle 1) of the Petrifilm Lactic Acid Bacteria Count Plate or from trapped air within the sample. They are irregularly shaped and not associated with a red colony. Do not count colonies on the dam.

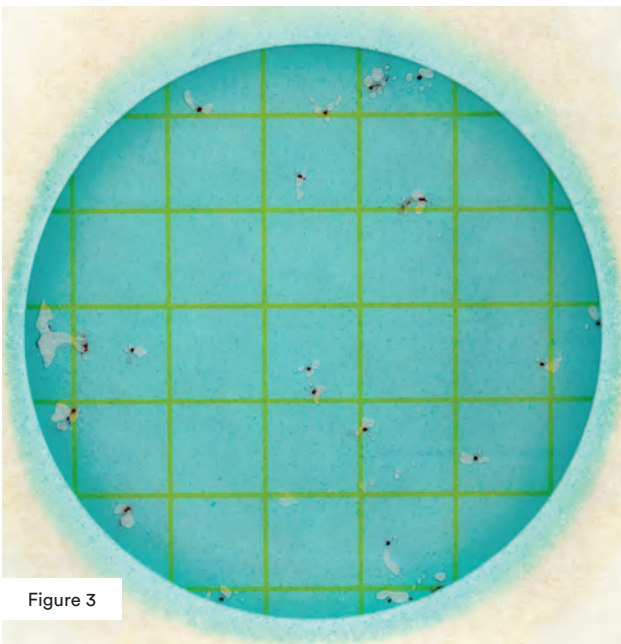


Figure 3

**Total lactic acid bacteria count = 24**

The recommended counting range becomes less than 150 colonies when colonies with gas are present. Gas bubble size will vary by size and shape. Gas may disrupt the colony so that the colony "outlines" the bubble.

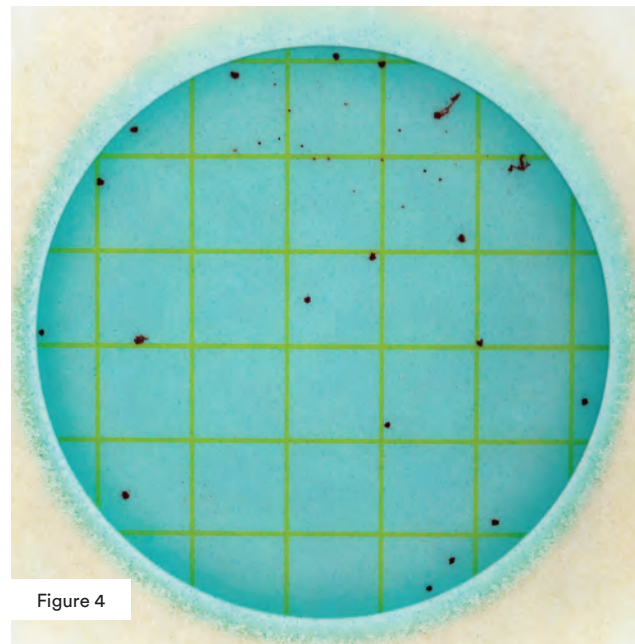


Figure 4

**Total lactic acid bacteria count = 38**

Count all colonies regardless of size and intensity.

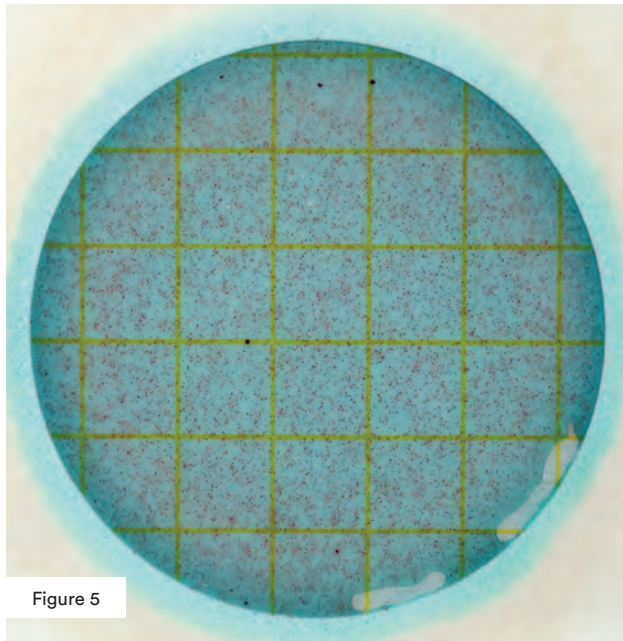


Figure 5

**Total lactic acid bacteria count = TNTC**

Petrifilm Lactic Acid Bacteria Count Plates with colony counts too numerous to count (TNTC) may have one or more of the following characteristics: many small colonies, many gas bubbles, and a deepening of the gel color from blue to pink-purple. High concentrations of colonies on the plates will cause the entire growth area to become deep-blue to purple with a pink halo around the outer edge of the plate.

*For a more accurate count, further dilution of the sample may be necessary.*

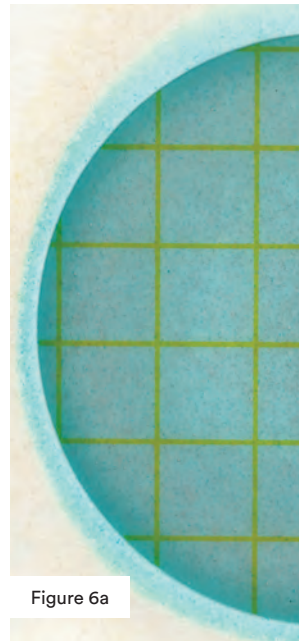


Figure 6a

**Total lactic acid bacteria count = TNTC**

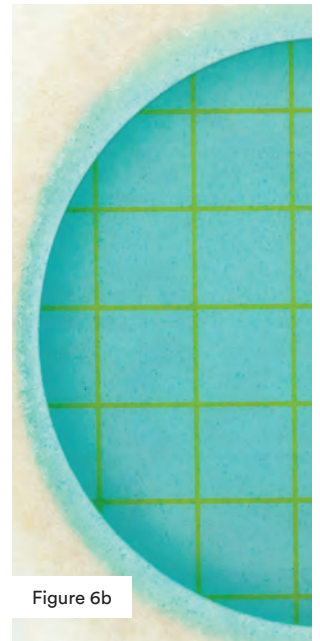


Figure 6b

**Total lactic acid bacteria count = 0**

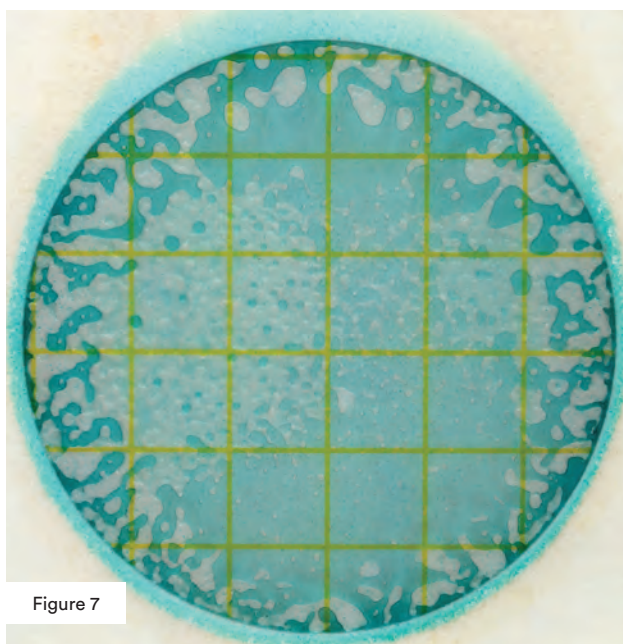


Figure 7

**Total lactic acid bacteria count = TNTC**

High concentrations of gas producing (heterofermentative) colonies on the Petrifilm Lactic Acid Bacteria Count Plates will result in an irregular distribution of many gas bubbles.

*For a more accurate count, further dilution of the sample may be necessary.*

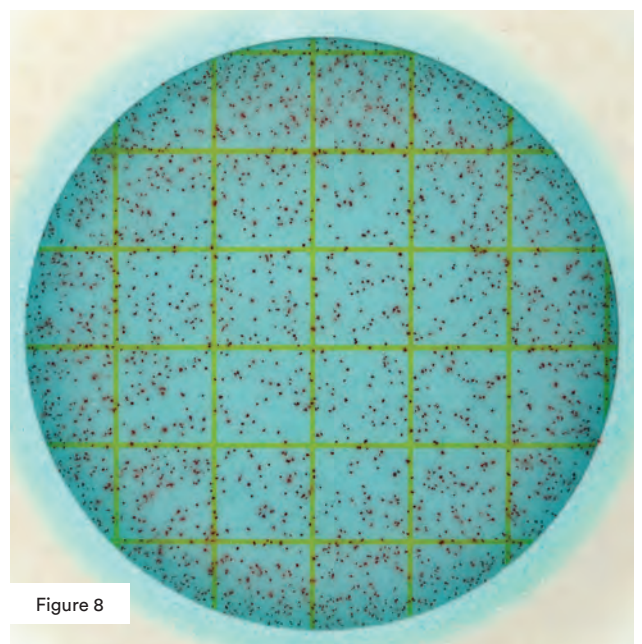


Figure 8

**Estimated total lactic acid bacteria count = 1500**

When the number of colonies without gas number more than 300, estimate the count. Determine the average number of colonies in two or more representative squares and multiply by 30 to obtain the estimated total count per plate. The inoculated area on a Petrifilm Lactic Acid Bacteria Count Plate is approximately 30cm<sup>2</sup>.

*For a more accurate count, further dilution of the sample may be necessary.*

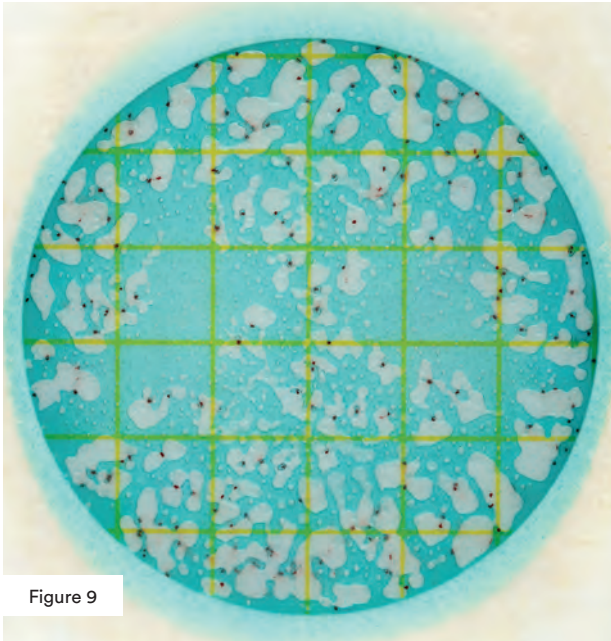


Figure 9

**Estimated total lactic acid bacteria count = 250**

When the number of colonies with gas number more than 150, estimate the count. Determine the average number of colonies in two or more representative squares and multiply by 30 to obtain the estimated total count per plate. The inoculated area on a Petrifilm Lactic Acid Bacteria Count Plate is approximately 30cm<sup>2</sup>.

*For a more accurate count, further dilution of the sample may be necessary.*

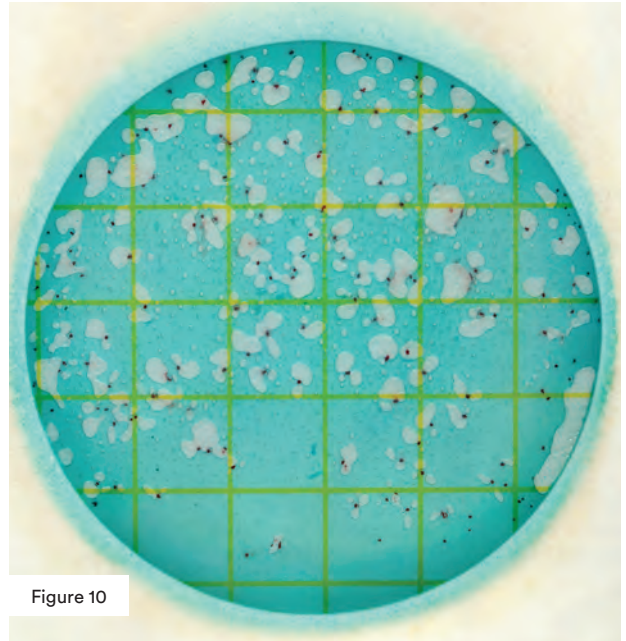


Figure 10

**Estimated total lactic acid bacteria count = 165**

Petrifilm Lactic Acid Bacteria Count Plates having greater than 150 colonies with and without gas, should be estimated. Determine the average number of colonies in two or more representative squares and multiply by 30 to obtain the estimated total count per plate. The inoculated area on a Petrifilm Lactic Acid Bacteria Count Plate is approximately 30cm<sup>2</sup>.

*For a more accurate count, further dilution of the sample may be necessary.*

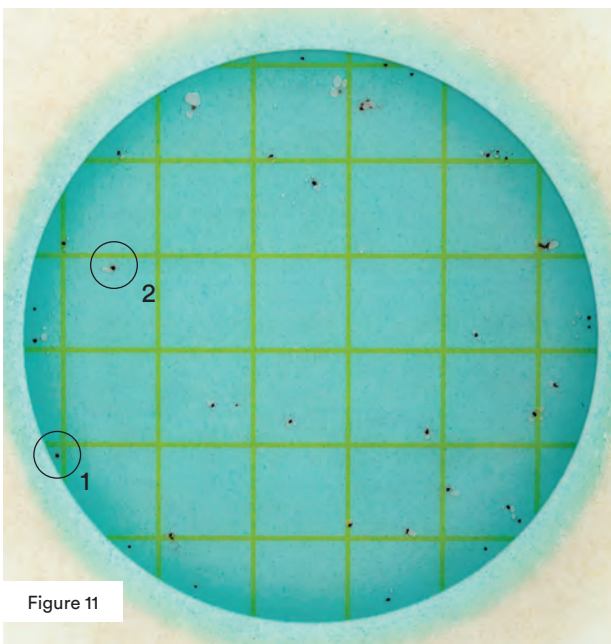


Figure 11

**Total lactic acid bacteria count: 41**  
**Homofermentative: 13 Heterofermentative count: 28**

Petrifilm Lactic Acid Bacteria Count Plate is able to differentiate homofermentative lactic acid bacteria from heterofermentative lactic acid bacteria. Heterofermentative lactic acid bacteria (Circle 2) are defined as colonies that are red and closely associated (within one colony diameter) with entrapped gas. Red colonies without gas (Circle 1) are defined as homofermentative lactic acid bacteria.

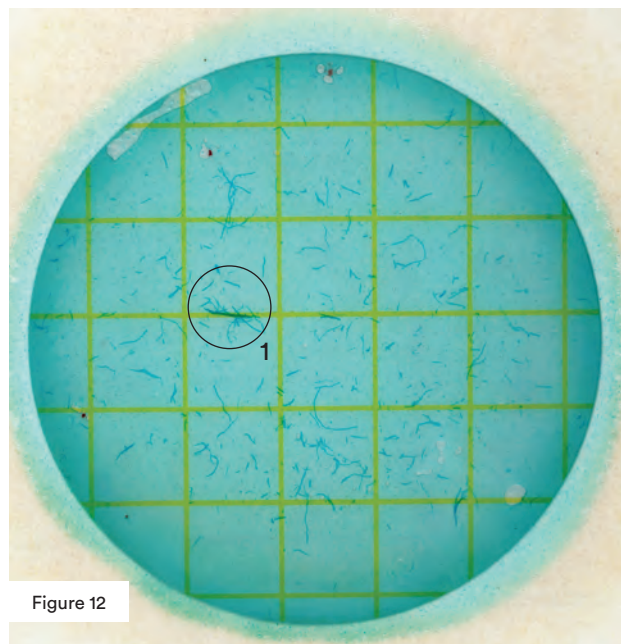


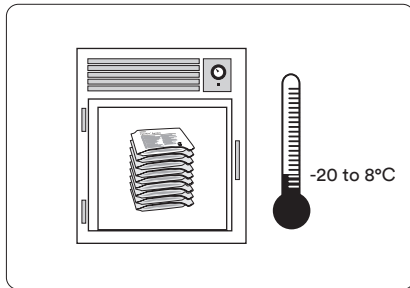
Figure 12

**Total lactic acid bacteria count: 4**  
**Homofermentative count: 1 Heterofermentative count: 3**

Food particles (Circle 1) are irregularly shaped or filamentous. Do not enumerate food particles. Artifact bubbles may result from improper inoculation of the Petrifilm Lactic Acid Bacteria Count Plate or from trapped air within the sample. They are irregularly shaped and not associated with a red colony. Do not enumerate artifact bubbles.

# Reminders For Use

## Storage



**01**

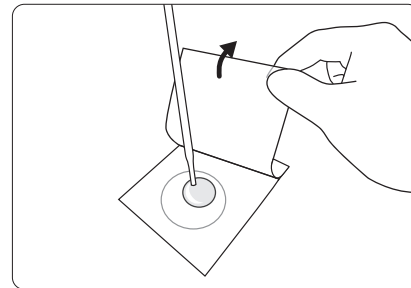
Store the unopened Petrifilm Lactic Acid Bacteria Count Plate pouches at frozen or refrigerated temperature equal to -20 to 8°C (-4 to 46°F). Use before expiration date on package. It is best to allow pouches to reach room temperature before opening.



**02**

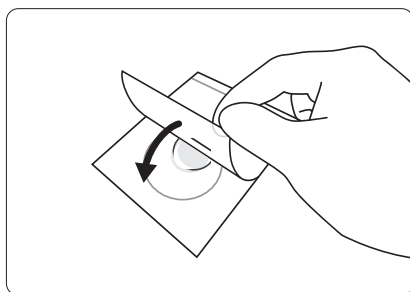
Seal by folding the end of the pouch over and applying adhesive tape. To prevent exposure to moisture, do not refrigerate opened pouches. Store sealed pouches in a cool dry place (20–25°C/<60% RH) or in a freezer ≤ -15°C (5°F) for no longer than four weeks.

## Inoculation



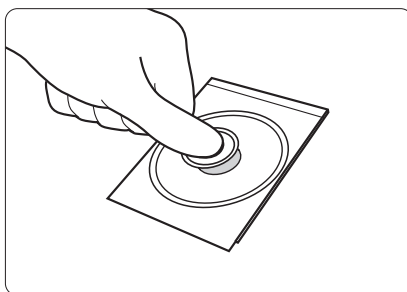
**03**

Place the Petrifilm Lactic Acid Bacteria Count Plate on a level surface. Lift the top film and, with the pipette **perpendicular** to the inoculation area, dispense 1mL of sample suspension onto the center of the bottom film.

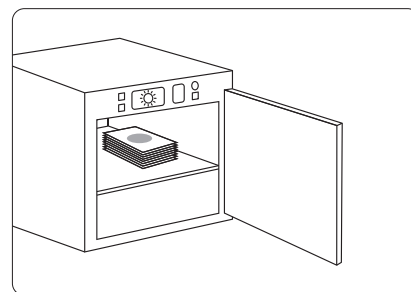


**04**

Roll the top film down onto the sample to prevent trapping air bubbles. Place the Petrifilm Flat Spreader on the center of the Petrifilm Lactic Acid Bacteria Count Plate. Press **gently** on the center of the Petrifilm Flat Spreader to distribute the sample evenly. Remove the Petrifilm Flat Spreader and leave the Petrifilm Lactic Acid Bacteria Count Plate undisturbed for at least one minute to permit the gel to form.



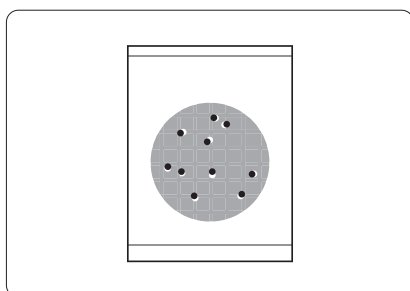
## Incubation



**05**

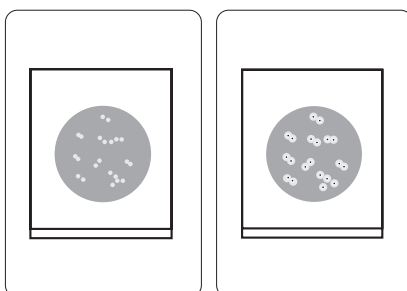
Incubate plates with clear sides up in stacks of no more than 20. Incubate the Petrifilm Lactic Acid Bacteria Count Plates for 48h ± 3h at 28–37°C. **Please refer to the product instructions for third party validated methods.**

## Interpretation



**06**

Petrifilm Lactic Acid Bacteria Count Plates can be counted using the Petrifilm Plate Reader Advanced, on a standard colony counter or other illuminated magnifier. Count all red colonies regardless of size or intensity. Do not count colonies on the dam since they are removed from the selective influence of the medium.



**07**

Heterofermentative lactic acid bacteria are defined as colonies that are red and closely associated (within one colony diameter) with entrapped gas. Red colonies without gas are defined as homofermentative lactic acid bacteria.

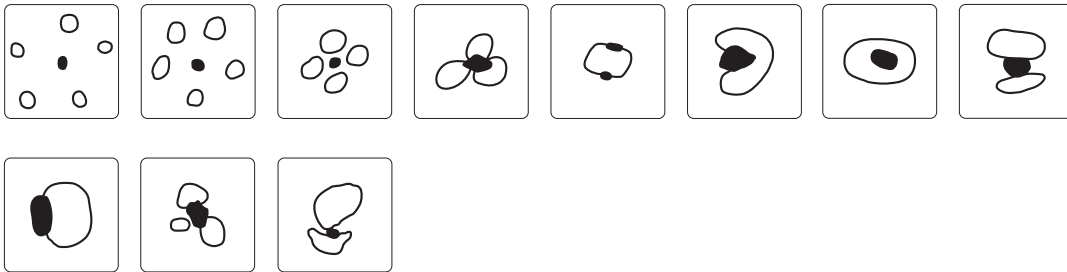
## Use Appropriate Sterile Diluents

Butterfield's phosphate-buffered dilution water, buffered peptone water, 0.1% peptone water, saline (0.85–0.90%), Wide-Spectrum Neutralizer, letheen broth, modified letheen broth or peptone salt diluent (Maximum Recovery Diluent).

**Do not use diluents containing citrate, bisulfite or thiosulfate with the Petrifilm Lactic Acid Bacteria Count Plates; they can inhibit growth.**

# Bubbles

The illustrations below show examples of various bubble patterns associated with gas producing colonies. It is possible to see more than one bubble pattern on one Petrifilm Lactic Acid Bacteria Count Plate. Images below should be enumerated as one colony.



Images below should be enumerated as two colonies.



Neogen offers a full line of products to accomplish a variety of your microbial testing needs.

For more product information, visit [info.neogen.com/petrifilm](https://info.neogen.com/petrifilm)

**User's Responsibilities:** Neogen Petrifilm Plate performance has not been evaluated with all combinations of microbial flora, incubation conditions and food matrices. It is the user's responsibility to determine that any test methods and results meet the user's requirements. Should re-printing of this Interpretation Guide be necessary, user's print settings may impact picture and color quality.

For detailed CAUTIONS, DISCLAIMER OF WARRANTIES/LIMITED REMEDY and LIMITATION OF NEOGEN LIABILITY, STORAGE AND DISPOSAL information and INSTRUCTIONS FOR USE, see product instructions.



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