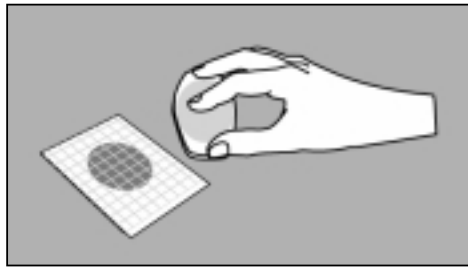


82-4000

# 3M<sup>™</sup> Petrifilm<sup>™</sup> Aerobic Count Plates

Instruction Manual



## Description

The 3M™ Petrifilm™ Aerobic Count (AC) Plate is a ready-made culture medium system that contains nutrients, a cold-water-soluble gelling agent, and an indicator dye that makes colonies easier to see. Petrifilm plates are manufactured with a grid background to facilitate counting colonies. Petrifilm AC plates can be used in place of standard nutrient media such as Luria broth agar plates, nutrient agar plates, or trypticase soya agar plates in many applications:

- Petrifilm plates can be hydrated with a bacterial culture, or a dilution of a culture, for counting the viable organisms present. See Method A.
- Petrifilm plates can be hydrated first with water or buffer, then inoculated by swabbing, streaking, or touching to surfaces. See Method B.
- Antibiotics can be added to the hydration fluid to select for resistant organisms in Method A or B.
- Cells can be removed from colonies growing on Petrifilm plates and used to inoculate additional cultures or for staining.
- Experimental results on Petrifilm plates can be saved for future reference by scanning the plates on a standard computer-linked scanner. Do not open the plates for scanning.

Petrifilm plates were developed for use in the food and beverage industry. They have been certified for official analyses in many countries. For more information about these applications, see the 3M page on the World Wide Web at [www.3m.com](http://www.3m.com).

## Storage

**Refrigerate unopened packages at  $\leq 8^{\circ}\text{C}$  ( $\leq 46^{\circ}\text{F}$ ).** Use before expiration date on package.

To seal an opened package, fold the end over and tape it shut (fig. 1). Keep resealed packages at room temperature and less than 50% relative humidity.

**Do not refrigerate opened packages.** Use plates from the opened package within one month after opening.



Figure 1

## Directions for Use

Use sterile technique when handling Petrifilm plates. Disinfect the work area by wiping thoroughly with alcohol or other disinfectant before and after use. Used Petrifilm plates may contain viable organisms. Do not open the plates unnecessarily.

### Method A. Inoculation with liquid sample

1. If a Petrifilm plate pack has been stored in the refrigerator, let the package come to room temperature before opening it. This step prevents condensation from forming inside the package.
2. Place the Petrifilm plate on a level surface, with the grid side down. Lift the top film (fig. 2).

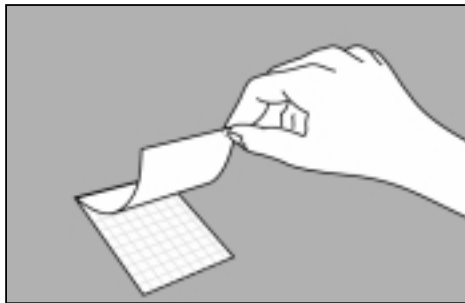
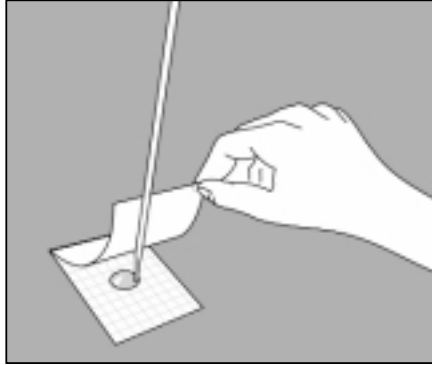


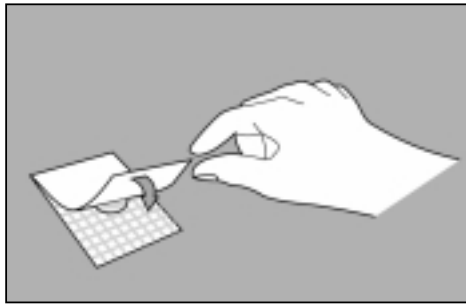
Figure 2

3. With pipet perpendicular to the Petrifilm plate, place 1 mL of sample onto the center of the bottom film (fig. 3). If necessary, samples can be diluted with distilled water, liquid culture medium, or buffers with pH between 6.6 and 7.2.\* If antibiotics are to be added to the medium, add them to the inoculating liquid at the working concentration.



**Figure 3**

4. Release the top film. Allow it to **drop** onto the bottom film. Do not roll the top film down (fig. 4).



**Figure 4**

5. Hold the spreader with the circular ridge down (flat side up). Place the spreader on the top film over the inoculum (fig. 5).

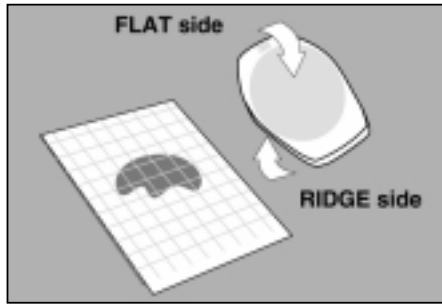


Figure 5

6. **Gently** apply pressure on the spreader to distribute the inoculum over a circular area. Do not twist or slide the spreader (fig. 6).



Figure 6

7. Lift the spreader. Wait at least one minute for the gel to solidify (fig. 7).

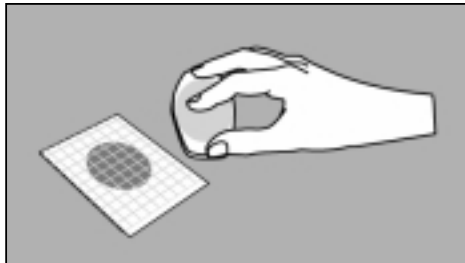
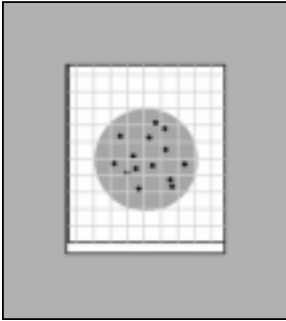


Figure 7

8. Incubate plates with the clear side up in stacks of up to 20 plates. Incubation time and temperature will vary according to the application and equipment available.
9. Colonies on Petrifilm plates can be counted on a standard colony counter or other light source (fig.8). Bacterial colonies on Petrifilm AC plates are red because of the indicator dye in the medium.



**Figure 8**



**Figure 9**

10. Colonies may be isolated for further study or to inoculate additional cultures. Lift the top film and pick the colony from the gel. The medium will adhere to the top film.
11. Disinfect before disposal. Petrifilm plates can be disinfected by autoclaving or by soaking in 20% bleach for one hour. Then, they can be placed in the trash. Open the plates in the bleach to expose organisms on the plates to the solution. Alternatively, they can be taken to a facility such as a hospital or given to a school nurse for disposal with other biohazardous material.

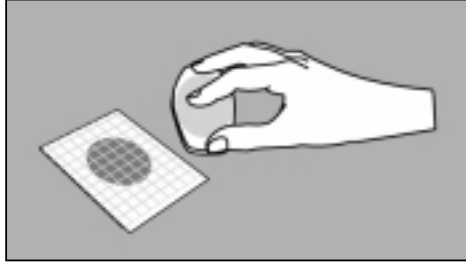
\*Do not use diluents that contain citrate or sodium thiosulfate, because they can inhibit growth on the Petrifilm plate. These substances are not found in common microbiological media such as Luria broth or nutrient broth.

### **Method B. Hydrating and using as solid medium**

Hydrate and spread each Petrifilm plate before going on to the next plate.

1. Follow steps 1–6 from Method A, using 1 mL of distilled water, liquid culture medium, or buffers with pH between 6.6 and 7.2 to hydrate the Petrifilm plate.\* If antibiotics are to be added to the medium, add them to the hydration liquid at the working concentration.

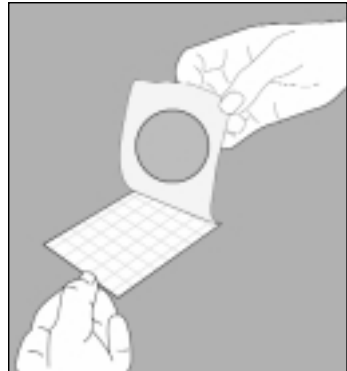
2. Lift the spreader. Wait at least 2 hours for the gel to solidify (fig. 10).



**Figure 10**

3. Hydrated Aerobic Count plates can be stored in the refrigerator in a sealed bag for up to 14 days before use.
4. To inoculate the medium, lift the top film. The circular gel area will adhere to the top film (fig. 11). Hydrated Petrifilm can be used in many ways:

- Streak for isolated colonies with a sterile loop, more gently and with less pressure than you would a standard plate. Tape the Petrifilm plate to a flat surface in the open position for streaking.
- Touch the circular gel area to a surface of interest. This could be a bench top, finger, doorknob, or other smooth object.
- Sample the air by peeling back the top film with the circular gel area and taping the open Petrifilm plate



**Figure 11**

to a vertical surface, so that the gel area is exposed. After exposure time has elapsed, take down the Petrifilm plate, close, and incubate.

Bacterial colonies on Petrifilm AC plates are red because of the indicator dye in the medium. The red color helps to distinguish them from dust particles or other environmental contaminants. Colonies may be isolated for further study or to inoculate additional cultures (see Method A, step 10).

\*Do not use diluents that contain citrate or sodium thiosulfate because they can inhibit growth on the Petrifilm plate. These substances are not found in common microbiological media such as Luria broth or nutrient broth.

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