

Product Instructions

-  **(EN)** *Bacillus cereus* Count Plate
-  **(FR)** Plaque de numération de *Bacillus cereus*
-  **(DE)** *Bacillus cereus* Zählplatte
-  **(IT)** Piastra per il conteggio di *Bacillus cereus*
-  **(ES)** Placa de recuento de *Bacillus cereus*
-  **(NL)** *Bacillus cereus*-tellingplaat
-  **(SV)** Odlingsplatta för antalsbestämning av *Bacillus cereus*
-  **(DA)** *Bacillus cereus* tælleplade
-  **(NO)** *Bacillus cereus* dyrkningsfilm
-  **(FI)** *Bacillus cereus* kasvatusalusta pesäkkeiden laskentaan
-  **(PT)** Placa de contagem *Bacillus cereus*
-  **(EL)** Πλακίδιο Καταμέτρησης *Bacillus cereus*
-  **(PL)** Płytko do oznaczania liczby *Bacillus cereus*
-  **(RU)** Тест-пластина для подсчета *Bacillus cereus*
-  **(TR)** *Bacillus cereus* Sayım Plakası
-  **(JA)** セレウス菌測定用プレート
-  **(ZH)** 蜡样芽胞杆菌测试片
-  **(TH)** แผ่นอาหารเลี้ยงเชื้อสำหรับนับจำนวนบาซิลลัส ซีเรียส
-  **(KO)** 바실러스 세레우스 카운트 플레이트

BC

Bacillus cereus

Product Instructions

Bacillus cereus Count Plate

Product Description and Intended Use

The Neogen® Petrifilm® *Bacillus cereus* Count (BC) Plate is a selective and differential sample-ready-culture-medium system which contains proprietary nutrients, a cold-water-soluble gelling agent, chromogenic indicators and a lecithinase substrate that facilitates colony enumeration.

This medium is used for the enumeration of the *Bacillus cereus* group, also known as the *B. cereus sensu lato* group, without confirmation in the food and beverage industries. This also includes, but is not limited to, the detection of *B. cytotoxicus*. The Neogen Petrifilm BC Plate components are decontaminated though not sterilized. Neogen Food Safety is certified to International Organization for Standardization (ISO) 9001 for design and manufacturing. The Neogen Petrifilm BC Plate has 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 Neogen Petrifilm BC 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:

- After use, Neogen Petrifilm BC Plates may contain microorganisms that may be a potential biohazard. Follow current industry standards and local, regional, and national requirements for disposal.

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

- Perform the Neogen Petrifilm BC 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 7218², or CDC Biosafety in Microbiology and Biomedical Laboratories (BMBL)¹².
- Isolates that are suspected to be *B. anthracis* should be destroyed by autoclaving or submitted to a designated pathology laboratory (for instance, in the United States, the Centers for Disease Control and Prevention, Atlanta, GA) for identification.

To reduce the risks associated with misinterpretation of results:

- Neogen has not documented the Neogen Petrifilm BC Plates for use in industries other than food and beverage. For example, Neogen has not documented the Neogen Petrifilm BC Plates for testing water, pharmaceuticals, or cosmetics.
- Acceptance of the Neogen Petrifilm BC Plate method for the testing of water per an accepted local government regulation is at the sole discretion and responsibility of the end user.
- Do not use the Neogen Petrifilm BC Plates in the diagnosis of conditions in humans or animals.
- The Neogen Petrifilm BC Plates do not differentiate any one *Bacillus cereus sensu lato* strain from another.

For information on documentation of product performance, visit our website at www.neogen.com or contact your Neogen representative or authorized Neogen distributor.

User Responsibility

Users are responsible for familiarizing themselves with product instructions and information. Visit our website at www.neogen.com, or contact your Neogen representative or authorized Neogen distributor for more information.

When selecting a test method, it is important to recognize that external factors such as sampling methods, testing protocols, food sample, 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 Neogen 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, NEOGEN DISCLAIMS ALL EXPRESS AND IMPLIED WARRANTIES, INCLUDING BUT NOT LIMITED TO, ANY WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR USE. If any Neogen Food Safety Product is defective, Neogen 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 Neogen within sixty days of discovery of any suspected defects in a product and return it to Neogen. Please call Customer Service or your official Neogen Food Safety representative for a Returned Goods Authorization.

Limitation of Neogen Liability

NEOGEN 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 NEOGEN'S LIABILITY UNDER ANY LEGAL THEORY EXCEED THE PURCHASE PRICE OF THE PRODUCT ALLEGED TO BE DEFECTIVE.

Storage

Store the unopened Neogen Petrifilm BC Plate pouches refrigerated or frozen at temperatures (-20 to 8°C/-4 to 46°F). Just prior to use, allow the unopened Neogen Petrifilm BC Plate pouches to come to room temperature before opening (20-25°C (68-77°F)/<60% RH). Return the unused Neogen Petrifilm BC 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 Neogen Petrifilm BC Plate pouches in a cool dry place for no longer than four weeks. It is recommended that resealed pouches of Neogen Petrifilm BC Plates be stored in a freezer for no longer than four weeks 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 the Neogen Petrifilm BC Plates in a sealable container. To remove the frozen Neogen Petrifilm BC Plates for use, open the container, remove the plates that are needed, and immediately return the remaining plates to the freezer in the sealed container for no longer than four weeks. The freezer that is used for open pouch storage must not have an automatic defrost cycle as this would repeatedly expose the Neogen Petrifilm BC Plates to moisture, which can damage the plates.

Do not use Neogen Petrifilm BC Plates that show discoloration. Use by date and batch code are noted on each package of the Neogen Petrifilm BC Plates. The lot number is also noted on individual Neogen Petrifilm BC Plates. The Neogen Petrifilm BC Plates should not be used past their expiration date.

⚠ Disposal

After use, Neogen Petrifilm BC Plates may contain microorganisms that may be a potential biohazard. Follow current industry standards and local, regional, and national requirements 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:

Butterfield's phosphate buffer⁴, 0.1% peptone water, peptone salt diluent⁵, phosphate buffered saline, buffered peptone water⁵, saline solution (0.85-0.90%), bisulfite-free letheen broth, or distilled water.

Do not use diluents containing citrate, bisulfite, or thiosulfate with the Neogen Petrifilm BC Plates; they can inhibit growth. See section "Specific Instructions for Validated Methods" for specific requirements.

2. Blend or homogenize the sample.
3. For optimal growth and recovery of microorganisms in acidic products (<pH 5), adjust the pH of the sample suspension to a pH greater than pH 5 using 1N NaOH.

Plating

1. Place the Neogen Petrifilm BC 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 Neogen® Petrifilm® Flat Spreader (6425) with the flat side down on the center of the plate. Press gently on the center of the spreader to distribute the sample evenly. Spread the inoculum over the entire Neogen Petrifilm BC Plate growth area before the gel is formed. Do not slide the spreader across the film.
5. Remove the Neogen Petrifilm Flat Spreader and leave the plate undisturbed for at least one minute to permit the gel to form.

Incubation

Incubate the Neogen Petrifilm BC Plates in a horizontal position with the clear side up in stacks of no more than 10 plates.

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. The Neogen Petrifilm BC Plates can be counted on a benchtop or 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.
2. Count all red-violet colonies with a cream/white precipitate around the colony as *Bacillus cereus* group. These typical colonies may or may not be associated with a yellow acid zone.
3. Certain strains of *Bacillus cytotoxicus* may occasionally exhibit a darker red-purple color with a distinct white precipitate at 30°C±1°C, while exhibiting typical morphology and appearance at 35°C±1°C.
4. Non-*Bacillus cereus* organisms will be inhibited or may appear as blue colonies or pinpoint red colonies with no cream/white precipitate around the colony.
5. In rare instances, non-*Bacillus cereus* group organisms such as *Bacillus inaquosorum* may form a small/pinpoint colony and express a white zone. Even if pinpoint colonies are not typical, and shouldn't be considered as *Bacillus cereus* group, the user may choose to perform further identification according to ISO 7932.
6. The circular growth area is approximately 30 cm². The counting range for Neogen Petrifilm *B. cereus* plate is lower than or equal to 100 red-violet colonies with a cream/white precipitate. Estimates can be made on the Neogen Petrifilm BC Plates containing greater than 100 colonies. Count the number of colonies in one or more representative squares and determine the average number per square. Multiply the average number by 30 to determine the estimated count per Neogen Petrifilm BC Plate.
7. High concentrations of *Bacillus cereus* on the Neogen Petrifilm BC Plates may appear as many small, indistinct colonies and/or darkening of the outer edge. When this occurs, record results as TNTC. For a more accurate count, further dilution of the sample may be necessary.
8. Where necessary, colonies may be isolated for further identification. Lift the top film and pick the colony from the gel. Test using standard procedures such as those described in FDA BAM Ch. 14, USDA FSIS MLG Ch. 12, or ISO 7932:2004.
9. If the Neogen Petrifilm BC Plates cannot be counted within the incubation period, they may be stored for later enumeration by freezing in a sealable container at temperatures lower than or equal to -15°C (5°F) for no longer than one week.

For further information refer to the “Neogen® Petrifilm® *Bacillus cereus* Count Plate Interpretation Guide”. If you have questions about specific applications or procedures, visit our website at www.neogen.com or contact your Neogen representative or authorized distributor.

Specific Instructions for Validated Methods

AOAC Certification:

AOAC® *Performance Tested*SM Certificate #042502 and AOAC® *Official Method of Analysis*SM 2025.01 are in compliance with AOAC Appendix J⁸ in comparison to BAM Chapter 14 (2020)⁹, MLG Chapter 12 (1998)¹¹ and ISO 7932:2004/Amd 1:2020⁶

Scope of the AOAC validation: Broad range of foods and selected pet foods.

The following procedures were followed:

In AOAC® *Official Methods of Analysis*SM and AOAC® *Performance Tested Methods*SM studies, the Neogen Petrifilm *Bacillus cereus* Count Plate method was found to be equivalent compared to the U.S. Food and Drug Administration *Bacteriological Analytical Manual (BAM)* Chapter 14, U.S. Department of Agriculture Food Safety and Inspection *Microbiology Laboratory Guidebook (MLG)*, and the International Organization for Standardization (ISO) 7932:2004/Amd 1:2020 methods for the enumeration of *Bacillus cereus* in the following sample types: non-fat dry milk, dairy-based ranch salad dressing, vanilla ice cream, ground black pepper, creamy peanut butter, rice flour, raw vegetable salad (cabbage, carrots, mayonnaise), dehydrated soup mix (minestrone), toddler puree (carrot, pear, pomegranate, oat), cooked turkey filet, chicken liver pate, cooked ready-to-eat chicken, infant rice cereal, infant formula with probiotics, infant formula without probiotics, cat pate, and dry dog kibble.

Sample preparation:

Prepare a 1:10 dilution by adding sample to the appropriate sterile diluent (up to 450 mL) Butterfield's phosphate buffer^{4,9} or MRD, also known as peptone salt diluent⁵.

Blend or homogenize the sample for 2 min in laboratory blender bags.

Incubation:

Incubate Petrifilm BC Plates 20-24 hours at 30°C±1°C or 35°C±1°C.



For more information about end of AOAC PTM validity, please refer to AOAC certificate.

MicroVal Certification:

MicroVal certificate number 2024LR132 in compliance with ISO 16140-2⁷ in comparison to ISO 7932:2004/Amd 1:2020⁶

Scope of the MicroVal validation:

Broad range of foods and animal feed

The following procedures were followed:

Sample preparation:

Use ISO listed diluents and prepare the samples according to ISO 6887 series⁵.

Incubation:

Incubate Petrifilm BC Plates 20–24 hours at 30°C±1°C.

Precautions:

Use good microbiology laboratory practices, such as ISO 7218. Enumeration using a single plate and a single dilution is an option certified in the MicroVal Certification. In this context, the requirements of ISO 7218 concerning the use of two successive dilutions or two plates of the same dilution cannot be applied (see paragraphs “Inoculation” and “Calculation and expression of results”). For sample preparation, follow ISO 6887 series including direct plating of liquids (milk and dairy alternatives) as specified in the standards. For more information about end of validity, please refer to MicroVal certificate.

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. 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 7932, Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of presumptive *Bacillus cereus* - Colony-count technique at 30 degrees C
7. ISO 16140-2. Microbiology of the food chain – Method Validation – Protocol for the validation of alternative (proprietary) methods against a reference method.
8. AOAC International (2012) *AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces*, Official Methods of Analysis, 22nd ed., Appendix J
9. U.S. Food and Drug Administration (2020) *Bacillus cereus. Bacteriological Analytical Manual*, Chapter 14 <https://www.fda.gov/food/laboratory-methods-food/bam-chapter-14-bacillus-cereus>.
10. U.S. Food and Drug Administration (2020) Food Sampling/Preparation of Sample Homogenate. *Bacteriological Analytical Manual*, Chapter 1 <https://www.fda.gov/food/laboratory-methods-food/bam-chapter-1-food-samplingpreparation-sample-homogenate>.
11. U.S. Department of Agriculture Food Safety and Inspection Service (1998) *Bacillus cereus. Microbiology Laboratory Guidebook*, Chapter 12 chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/ https://www.fsis.usda.gov/sites/default/files/media_file/2021-03/Mlgchp12.pdf
12. CDC Biosafety in Microbiological and Biomedical Laboratories (BMLB) 6th Edition. Effective Date: 29 August 2025.

Explanation of Symbols

info.neogen.com/symbols

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