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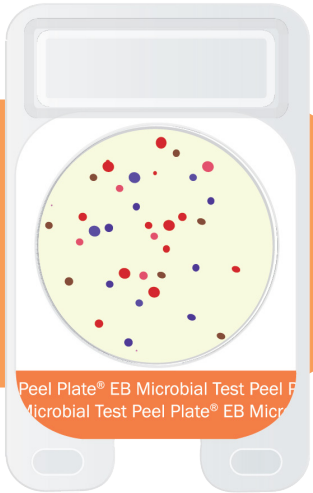


OPERATOR'S MANUAL

Peel Plate® EB ENTEROBACTERIACEAE

FOR DETECTION AND ENUMERATION OF ENTEROBACTERIACEAE BACTERIA IN FOOD, SERIAL DILUTIONS OF FOOD, AND ENVIRONMENTAL SPONGE SAMPLES

Contents

Kit Information

Introduction

Peel Plate EB (Enterobacteriaceae) tests detect and enumerate Enterobacteriaceae bacteria in food, serial dilutions of food, and environmental sponge samples (refer to Applicability for validated matrices). Sample or sample dilution is added and incubated for 24 to 48 hours at 37 °C ± 1 °C. Peel Plate EB tests are intended for microbiological laboratories, but may also be used by food quality stakeholders such as farmers, milk processors, and water municipalities. The method sensitivity is greater than 1 colony forming units per milliliter (>1 CFU/mL) of test sample. The accurate quantitative range is defined as 1 to 150 CFU/plate.

Kit Contents, Storage, and Testing Conditions

A test kit (item code: PP-EB-100K) contains 100 tests - 50 tests in two desiccated foil bags containing a blue indicator desiccant.

Kits are not required to be shipped refrigerated.

Store kits in foil bag refrigerated* or at room temperature, ambient temperature less than 25 °C, until expiration date.

Open bag and perform testing in a clean dry testing area at ambient temperature. Remove number of plates need for analysis. **Tests held at ambient temperature for 1 hour or more will open more easily.** Reseal the bag using the zip closure to store unused tests. Moisture, heat, or storage abused test will discolor yellow. Do not use discolored tests or tests from bags with a pink/white desiccant indicator.

*** Refrigeration is defined as 0 to 4.5 °C and is required for US Certified Labs**

Principle

The Peel Plate EB test is based on bile salt selective agar, glucose, and multiple colorimetric enzyme substrates to support growth and colorimetrically identify the growth of the family of enterobacteriaceae bacteria. The media also contains gelling and wicking agents which absorb and diffuse the sample.

Applicability

The Peel Plate EB test is applicable to multiple food matrices incubated in the dark at 37 °C ± 1 °C for 24 to 48 hours. The method has been validated according to ISO 16140-2 (2016) for certification by MicroVal for two food categories: 1-Heat processed milk and dairy products, and 2-infant formula and infant cereals. Test portions preparation was conducted in accordance with ISO 6887 parts 1, 4 and 5. The method is considered "First Action" under the AOAC Official Methods of Analysis (OMA) program for whole milk, powdered milk, infant formula (dairy based) with and without probiotic, soy based infant formula, butter, vanilla ice cream, rice infant cereal, sponges of stainless steel surfaces and chicken carcass rinse with buffered peptone water and neutralizing buffered peptone water. The Peel Plate EB method has been compared to a reference method, ISO 21528 parts 1 and 2 methods, and no significant differences were observed during the validations. The Peel Plate EB method may also be applicable to other non-cultured dairy products, meats, eggs, processed foods, pet foods and their contact surfaces but has not been officially validated by certification organizations.

Samples should be 10-fold serially diluted into the countable range of 1 to 150 CFU/mL.

Precautions

Observe Good Laboratory Practices for microbial testing. Avoid specimen contamination.

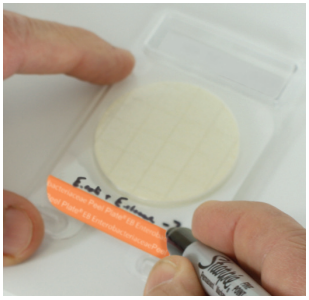
- Raw foods, processed foods, animal products, and their contact surfaces may contain harmful microorganisms or pathogens such as *Listeria monocytogenes*, hemorrhagic *E. coli*, and Salmonella.
- Take care in handling raw food and animal products, and the developed tests as they may contain these potential hazardous microorganisms.
- If direct contact or spillage occurs, thoroughly wash affected area using detergent and water for skin and clothing; and disinfectant for other surfaces. If eye or mouth contact rinse thoroughly. If there is a subsequent illness, irritation or infection contact a physician.
- Avoid contact with tests samples and Peel Plate EB medium. Perform tests and handle developed tests wearing personal protective equipment such as eye wear, lab coats and gloves.
- Perform test on a level surface in a clean area, free of dust and blowing air.
- After plating, re-seal adhesive cover so that it lays flat with no wrinkles to avoid drying out the rehydrated medium during incubation.
- To reduce the potential of sample cross contamination, wash and disinfect, glassware and work area in contact with foods and developed tests.

- Some cultured dairy products, such as cheeses, yogurts, cottage cheeses, sour cream, may produce a red background interference in the method. An alternative formulated test, specific for cultured dairy products, is available if interference from matrix is observed.

Sample Preparation

Liquid Food	• Liquid food samples (milk, pasteurized liquid dairy products, eggs) may be tested directly or serially diluted to a countable range (1 to 150 CFU/mL). ➤ To serially dilute, add 11 mL sample into 99 mL microbiologically suitable dilution blanks[A]. Other automated dilution pipets and 1 part sample to 9 part buffer dilution schemes are acceptable. • For milk powders and evaporated/condensed milks reconstitute to normal milk solids with sterile water and let settle 3 minutes. Test as liquid food. [A]Note - Validated matrixes use 0.1% peptone water as diluent.
Solid Food	• Add 25 g of solid food (ice cream, evaporated and sweetened condensed milk, non-cultured dairy products, ground beef, pet food, etc) to 225 mL[B] of microbiologically suitable dilution blank[A] and serially dilute as necessary to reach countable range (1 to 150 CFU/mL). • Homogenize or stomach for 2 minutes. Let particulates settle, and continue to dilute 10 mL of prior dilution in 90 mL (or 11 to 99 mL) of dilution blank to reach countable range. Other 1 part to 9 part dilution schemes are acceptable. [B]Note - For cereals, and other matrixes that may absorb diluent, it may require a 1:50 dilution, e.g. 25 g to 1225 mL to create a sample that will wick and spread into the Peel Plate. In this case 5 mL of the 1:50 dilution is equal to 1mL of 1:10 is equivalent. 5 mL may be tested in five Peel Plate EB tests or in one Peel Plate High Volume (HV) test, refer to Peel Plate EBHV operators manual.
Environmental Swab	• **Refer to Peel Plate Sample Preparation Addendum.**

Peel Plate EB Test Procedure

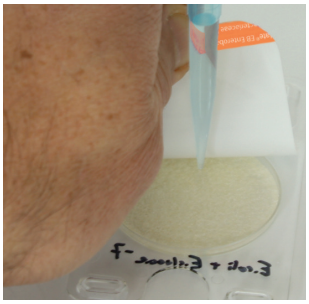


Step 1

- For ease of opening, use plates at room temperature.
- Label plate on clear side using marker or bar code strip. Do not mark or label the uplifted 47 mm circular area.
- For best results, hold plates at room temperature prior to plating.

Step 2

- Invert and place test onto a level surface. Apply pressure with fingers to the back platform as shown and lift tab.
- Pull the adhesive cover exposing the culture disc. Leave cover adhered to back of plate.



Step 3

- While holding cover up, and keeping plate flat on surface, **vertically dispense 1.0 mL onto the center of disc**. Expel in 2 to 3 seconds while 1 to 2 cm from surface.

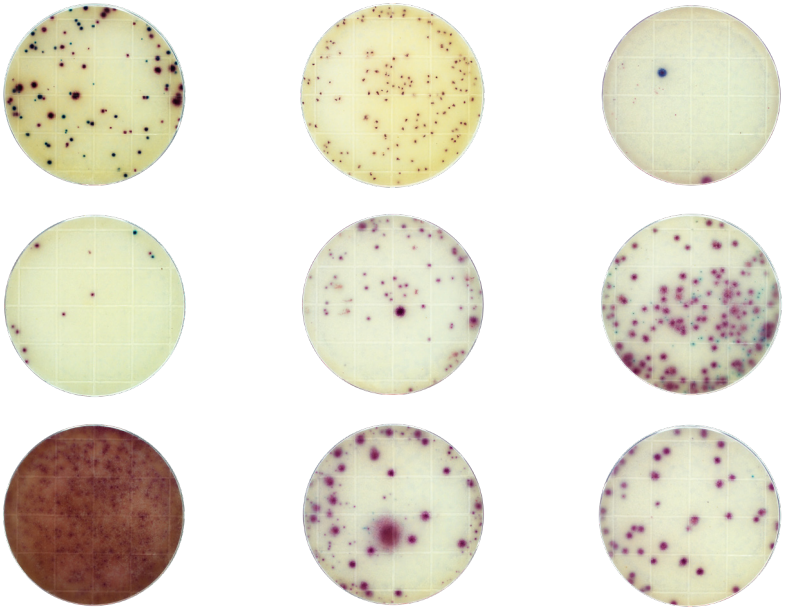
Step 4

- Sample will diffuse to the edges of the disc.
- Re-seal the adhesive cover without wrinkling. Press around edges of plate to ensure proper seal.

Step 5

- Incubate plates in the dark with clear side up, as shown. Incubate at **37 ± 1 °C for 24 to 48 hours**.
- Plates can stack up to 20 high by aligning the 2 feet and rectangular platform. Stacking will not affect plate heat transfer.

Analysis of Results



- At the end of incubation period, observe plates for colonies as viewed through the clear side of plate. Each spot regardless of color represents 1 CFU of EB. The sum of spots is reported as the CFU/mL or CFU/gram per dilution tested.

- In case of spreading bacteria, score a single CFU for each spot within the spread growth. Blended colonies are scored as a single CFU.
- Multiply CFU/mL by the dilution reciprocal to calculate a CFU/mL or CFU/g sample.
- Counts of 1 to 150 CFU/plate are considered quantitative results, while counts outside that range are considered estimates.
- Samples with results outside quantitative range should be diluted and retested.
- An estimated count of plates with greater than 150 colonies or Too Numerous To Count (TNTC) may be done using the etched grids. Pick a 1 cm grid with representative growth and count, or pick 5 grids and take average, and multiply by 17.4, the area of the plate. This is the estimate of the counts per plate. This would then be multiplied by the dilution factor for CFU/mL or g sample.

Quality Control

Quality control should be performed according to Good Laboratory Practices and with the frequency determined by laboratory standard operating procedures. Common practices call for a Dilution Control, Negative Control, and Positive Control.

- **Dilution Control:** Test 1.0 mL of sterile dilution buffer to verify no detectable bacteria on Peel Plate test after incubation.
- **Negative Control:** Prepare Negative Control by autoclaving the appropriate dilution of test sample at 121 °C for 15 minutes. Cool, then test 1.0 mL to verify no detectable bacteria in the Negative Control.
- **Positive Control:** Prepare Positive Control by spiking a sample with known titer bacterial culture. Dilute sample to countable range of 1 to 150 CFU/mL. Test 1.0 mL and verify detection after incubation to be within ± 50 % of estimated titer bacterial culture.

Disposal

Collect microbiological cultures and reagents in biohazard bags and autoclave. Dispose according to local, state, and federal regulations.

Technical Support

For questions, contact your local representative or Charm Sciences at +1.978.687.9200 or **support@charm.com.**

Order Information

Description	Quantity	Kit Code
Peel Plate EB	100	PP-EB-100K
	1000	PP-EB-1000K

Peel Plate tests for *E. coli* and coliforms, coliform count, aerobic bacteria, yeast and mold, and heterotrophic bacteria are also available. Visit Charm Sciences' website at www.charm.com to learn more.

Warranty

Charm Sciences, Inc. (“Charm”) warrants each reagent product, including but not limited to test kits, to be free from defects in materials and workmanship and to be free from deviations from the specifications and descriptions of Charm’s reagent products appearing in Charm’s product literature, when stored under appropriate conditions and given normal, proper and intended usage, until the expiration of such reagent product’s stated shelf life, or, if none is stated, for one year from the date of delivery of such reagent product to the end-user purchaser. THIS WARRANTY IS IN LIEU OF ALL OTHER WARRANTIES, WHETHER STATUTORY, EXPRESS, IMPLIED (INCLUDING WARRANTIES OF TITLE, NON-INFRINGEMENT, MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE AND ALL WARRANTIES ARISING FROM COURSE OF DEALING OR USAGE OF TRADE). The warranty provided herein may not be altered except by express written agreement signed by an officer of Charm. Representations, oral or written, which are inconsistent with this warranty are not authorized and if given, should not be relied upon. In the event of a breach of the foregoing warranty, Charm’s sole obligation shall be to replace any reagent product or part thereof that proves defective in materials or workmanship within the warranty period, provided the customer notifies Charm promptly of any such defect prior to the expiration of said warranty period. The exclusive remedy provided herein shall not be deemed to have failed of its essential purpose so long as Charm is willing to replace any nonconforming reagent product or part. Charm shall not be liable for consequential, incidental, special or any other indirect damages resulting from economic loss or property damages sustained by any customer from the use of its reagent products. Except for Charm’s obligation set forth above to replace any reagent product that proves defective within the warranty period, Charm shall not be liable for any damages of any kind arising out of or caused by any incorrect or erroneous test results obtained while using any such reagent product, whether or not caused by a defect in such reagent product.

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