

OPERATOR'S MANUAL



Peel Plate® EB-CD

ENTEROBACTERIACEAE
FORMULATION FOR CULTURED DAIRY

**FOR DETECTION AND ENUMERATION OF ENTEROBACTERIACEAE
BACTERIA IN CULTURED DAIRY PRODUCTS**

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Kit Information

Introduction

Peel Plate® Enterobacteriaceae (EB-CD) for cultured dairy products test detects and enumerates Enterobacteriaceae bacteria in 1 mL sample volumes. The Peel Plate EB-CD contains reagents to reduce background interference and facilitate bacterial detection in cultured dairy products. The method is applicable for detecting and distinguishing Enterobacteriaceae in cultured dairy products (with the exception of certain cheeses like feta and mozzarella that produce no background interference on a conventional Peel Plate EB test) when incubated at 37 °C for 24 to 48 hours. The Peel Plate EB-CD test is intended for microbiological laboratories, but may also be used by food quality stakeholders. The method sensitivity is 1 or greater colony forming units per milliliter or gram (CFU/mL or g) of the test sample. The accurate quantitative range for EB is defined as 1 to 150 CFU/plate.

Kit Contents, Storage, and Testing Conditions

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

Kits are not required to be shipped refrigerated.

Store kits in foil bag in refrigerator* for up to 12 months or at room temperature for up to 1 month.

Open foil bag and remove the number of plates needed for analysis. Perform testing in a clean, dry testing area at ambient temperature. Reseal the bag using the zip closure to store unused tests. Moisture, heat, or storage abused tests 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-CD 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 Enterobacteriaceae. Additionally, pH adjusting chemicals and a bisulfite selective agent is added to EB-CD test to reduce background interference from starter cultures used in cultured dairy products. Peel Plate EB-CD tests also contain gelling and wicking agents which absorb and diffuse the sample.

Applicability

Peel Plate EB-CD test has been validated for detection of Enterobacteriaceae in cultured dairy (sour cream, yogurt, cottage cheese, cheeses, etc.). Some cheese varieties like mozzarella and feta do not produce a background on the conventional Peel Plate EB formulation so conventional PP-EB may be used for Enterobacteriaceae determination. Samples should be 10-fold serially diluted into the countable range of 1 to 150 CFU/plate.

Precautions:

- Observe Good Laboratory Practices for microbial testing. Avoid specimen contamination.
- Perform tests with clean washed and gloved hands assuming potential pathogenic bacteria.
- Test on a level surface in a clean area, free of dust and draft.
- Avoid hand contact with test samples and Peel Plate EB-CD medium.

Sample Preparation

Dairy (Liquid Cultured Drinks)

- Liquid cultured dairy samples may be tested 1:10 or serially diluted to a countable range (1 to 150 CFU/mL).
- To serially dilute, add 11 mL into 99 mL microbiologically suitable dilution blanks. Other automated dilution pipets and dilution schemes are acceptable.

Solid Dairy

- Add 11 g of solid dairy (sour cream, yogurt, cottage cheese, cheeses, etc.) to 99 mL of microbiologically suitable dilution blanks to reach countable range (1 to 150 CFU/mL).
- For dried powders (e.g. Whey), reconstitute 1:10 with diluent and let any undissolved solids settle (no more than 3 minutes).

Environmental Swab

Refer to Peel Plate Sample Preparation Addendum.

Peel Plate EB-CD Procedure

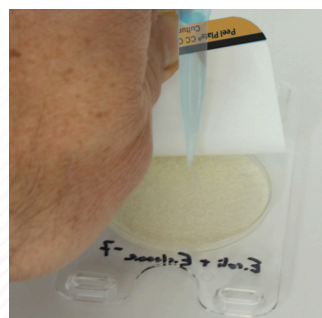
- Step 1
- Label plate on clear side using a marker. Do not mark or label the uplifted 47 mm circular area.

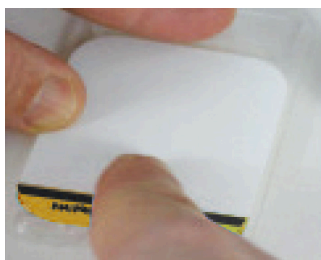


- Step 2
- For best results, hold plates at room temperature prior to plating.
 - Apply pressure to back side of plate and pull up the cover tab.
 - Lift cover to expose the Peel Plate EB-CD media.

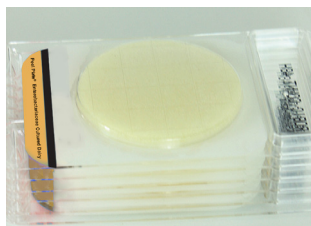


- Step 3
- Rapidly dispense 1.0 mL of sample, or sample dilution, onto the center of exposed plate from a distance of 1-2 cm. Expel pipet contents rapidly with even force and within 2 to 3 seconds.



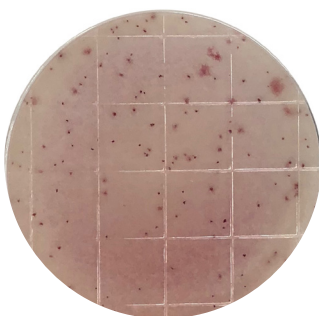


- Step 4
- Sample will diffuse towards the edges of plate. For viscous samples lift plate and rotate to ensure proper distribution of sample.
 - Re-apply the cover and smooth around the edges of the plate to seal the adhesive; avoid wrinkling.



- Step 5
- Incubate plates in the dark with clear side up, as shown.
 - Incubate at 37 °C for 24 to 48 hours for cultured products.
 - Plates can stack by aligning the feet and rectangular platform. Stacking plates up to 20 high will not affect plate heat transfer.

Analysis of Results



- At the end of the incubation period, observe plates for colonies through the clear side of the test. 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.
- Multiply CFU/mL by dilution reciprocal to calculate CFU/(mL or g) of original sample.

- In case of spreading bacteria, score one CFU for each defined spot. Blended or spreading colonies are scored as a single CFU.
- Counts of 1 to 150 CFU/plate are considered countable, while counts outside that range are considered estimates. Samples with results outside of countable range (>150 CFU/plate) can be diluted and retested.
- Too numerous to count results (TNTC) may be estimated by counting the colonies in a representative 1 square centimeter grid square, or taking an average of 5 cm², and multiplying by 17.4 for estimated colonies per plate (eCFU/plate).
- Cultured samples containing active LAB, e.g. cheeses, may present a reddish background. Background should be uniform and pink, not a dark red. Dark red would indicate TNTC and sample should be diluted to quantitate. It is recommended for a select number of cheeses like mozzarella and feta to use the conventional EB Peel Plate because those cheese types do not present a red background

Optional Colony Counter

- Insert completed test into the Colony Counter. Identify the plate as Peel Plate EB-CD.
- Enter sample identity or verify that bar code identity has been populated.
- Press COUNT and CFU/plate Enterobacteriaceae and, optionally CFU/mL Enterobacteriaceae, will be displayed and recorded into memory with time/date. For more information refer to Colony Counter instructions.

Product Claim Limitations

- EB-CD has been tested on many cultured dairy products like yogurt, cottage cheese, cheeses etc., but not all versions and flavors and types of cultured products. Products that produce a strong red background on the plate within a few hours of plate wicking may not be suitable for use.

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 after incubation.
- **Negative Control:** Prepare Negative Control by autoclaving the appropriate dilution of the test sample at 121 °C for 15 minutes. Cool to 4 °C and test 1.0 mL. Verify no detectable Enterobacteriaceae bacteria in the Negative Control.

- **Positive Control:** To prepare, spike a sample with known Enterobacteriaceae culture. Dilute the sample to countable range of 1 to 150 CFU/mL and test 1.0 mL to verify detection after incubation.

Disposal

Microbiological cultures and reagents should be collected in biohazard bags and autoclaved. Dispose according to local, state, and federal regulations.

Technical Support

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

Order Information

Peel Plate for Enterobacteriaceae in Cultured Dairy	100	PP-EB-CD-100K
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Peel Plate tests are also available in 5 mL sample volume tests and in 100 and 1000 test kits for Aerobic Count, *E.coli*/coliform, Enterobacteriaceae, Yeast/Mold and Heterotrophic Plate Count. Refer to www.charm.com for more information.

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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