

## OPERATOR'S MANUAL



**Peel Plate® CC-CD**  
COLIFORM COUNT CULTURED DAIRY

FOR DETECTION AND ENUMERATION OF TOTAL COLIFORM  
BACTERIA IN CULTURED DAIRY PRODUCTS



# Contents

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<b>Kit Information</b>	<b>4</b>
Introduction . . . . .	4
Kit Contents, Storage, and Testing Conditions . . . . .	4
Principle . . . . .	5
Applicability . . . . .	5
Precautions . . . . .	5
<hr/>	
<b>Sample Preparation</b>	<b>5</b>
Dairy (Liquid) . . . . .	5
Solid Dairy. . . . .	5
Environmental Swab . . . . .	6
<hr/>	
<b>Peel Plate CC-CD Procedure</b>	<b>6</b>
<hr/>	
<b>Analysis of Results</b>	<b>7</b>
<hr/>	
<b>Product Claim Limitations</b>	<b>8</b>
<hr/>	
<b>Quality Control</b>	<b>9</b>
<hr/>	
<b>Disposal</b>	<b>9</b>
<hr/>	
<b>Technical Support</b>	<b>9</b>
<hr/>	
<b>Order Information</b>	<b>9</b>
<hr/>	
<b>Warranty</b>	<b>10</b>

## Kit Information

### Introduction

Peel Plate® Coliform Count Cultured Dairy (CC-CD) test detects and enumerates total coliform bacteria in cultured dairy products like sour cream, cottage cheese, yogurt and cheeses that produce a red background on a Peel Plate CC test. The method is applicable for determination of total coliform in cultured dairy products when incubated at  $32 \pm 1$  °C for  $24 \pm 2$  hours. Peel Plate CC-CD tests are intended for microbiological laboratories, but may also be used by food quality stakeholders such as farmers, milk and food processors. The method limit of detection is 1 or greater colony forming units per milliliter or gram (CFU/mL or g) of test sample. The accurate quantitative range for coliform is defined as 1 to 154 CFU/plate.

### Kit Contents, Storage, and Testing Conditions

A test kit (item code PP-CC-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 CC-CD medium is based on conventional coliform selective medium to support and colorimetrically differentiate coliform in test samples. The Peel Plate CC-CD test contains the enzyme substrates salmon-gal (6-chloro-3-indolyl-B-D-galactopyranoside) used to detect  $\beta$ -galactosidase enzyme produced by coliform. Additionally the CC-CD test includes pH adjustment and background reduction agents, such as bisulfite, that are not incorporated in the CC test. The Peel Plate CC-CD test also contains gelling and wicking agents which absorb and self-diffuse the sample.

## Applicability

Peel Plate CC-CD test has been validated for detection of total coliform in cultured dairy (sour cream, cottage cheese, yogurt, cheeses) and found not significantly different from reference method Violet Red Bile Agar (VRBA) with colonies confirmed with Brilliant Green Lactose Bile (BGLB) broth. Samples should be 10-fold serially diluted into the countable range of 1 to 154 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 CC-CD medium.

## Sample Preparation

### Dairy (Liquid Cultured Drinks)

- Add 11 mL sample into 99 mL microbiologically suitable dilution blanks.
- Additional serial dilution schemes may be executed to achieve a countable range on the plate.

## Solid Dairy

- Add 11 g of solid dairy (cottage cheese, sour cream, yogurt, etc.) to 99 mL of microbiologically suitable dilution blanks to reach countable range (1 to 154 CFU/5mL). Mix/homogenize and let any undissolved solids settle (no more than 3 minutes).
- 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.

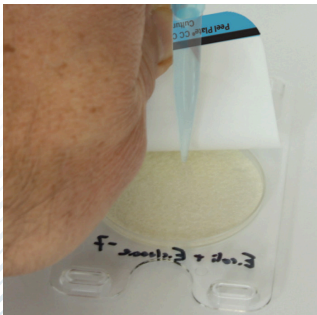
## Peel Plate CC-CD Procedure



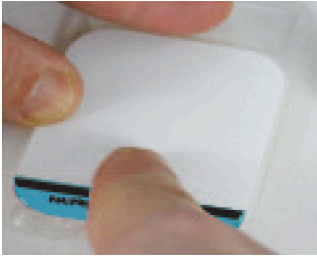
- Step 1
- Label plate on clear side using a marker. Do not mark or label the uplifted 47mm circular area.



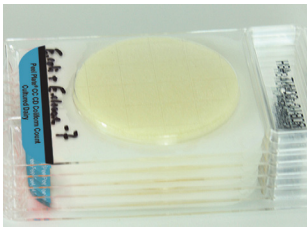
- Step 2
- For best results, hold plates at room temperature prior to plating.
  - Apply pressure to back side of plate and pull up the cover tab.
  - Lift cover to expose the Peel Plate CC-CD media.



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

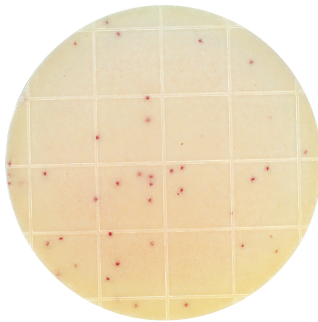


- Step 4
- Sample will diffuse towards the edges of plate. For viscous samples gently tilt the plate towards the uplifted cover and rotate to ensure proper distribution of sample.
  - Re-seal the adhesive cover without wrinkling. Press around edges of plate to ensure proper seal.
  - Allow gel to set for 10 seconds before moving plate.



- Step 5
- Incubate plates in the dark with clear side up, as shown.
  - Incubate at  $32 \pm 1$  °C for  $24 \pm 2$  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 distinct round red spot represents one CFU coliform. Coliform growth in cultured dairy products appears as round red colonies which may be surrounded by a reddish halo, though size may vary. The sum of spots is reported as the total coliform CFU/mL of the diluted sample.

- 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 154 CFU/plate are considered countable, while counts outside that range are considered estimates. Samples with results outside of countable range (>154 CFU/test) 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 cm<sup>2</sup>, and multiplying by 17.4 for estimated colonies per plate (eCFU/plate).
- Cultured samples containing active LAB, e.g. some yogurt, may present a reddish background.
- Any background should be consistent within a matrix. Reddish colonies should be distinguishable from any background in 24 hours.
- Do not count diffuse gray spots or non-round spots as coliform CFU. Red development and tiny red pinpoint growth on the edges of the plate should not be scored as coliform growth. These may be caused by freshly produced and actively growing LAB and their enzymes in cultured dairy product. If these occur call Charm Technical Support 1-800-343-2170 for suggestions and potential corrective actions.

### Optional Colony Counter

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

## Product Claim Limitations

- CC-CD has been tested on many fermented products like yogurt, cottage cheese, cheeses etc., but not all versions and flavors and types of fermented products. Products that produce a strong red background on the plate within a few hours of plate wicking may not be suitable for use.
- The Peel Plate CC-CD formulation is very similar to the Peel Plate EC formulation except the *E. coli* color indicator is removed and some pH adjustment and background reduction agents e.g. bisulfite, are incorporated into the formulation. In the Peel Plate EC test inclusivity and exclusivity studies, 57 of 58 coliform inclusivity isolates were correctly 9 detected, including all 17 *E. coli* strains. Of the 32 exclusivity strains evaluated, 31 were correctly excluded.



## 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 coliform bacteria in the Negative Control.
- **Positive Control:** To prepare, spike a sample with known coliform culture. Dilute the sample to countable range of 1 to 154 CFU/5 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](mailto:support@charm.com).

## Order Information

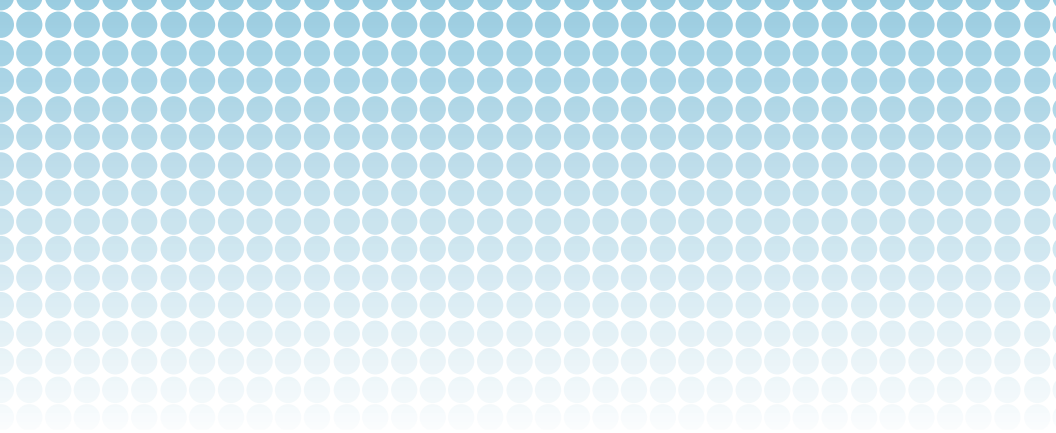
Peel Plate for Coliform in Cultured Dairy	100	PP-CC-CD-100K
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Peel Plate tests are also available in 5 mL sample volume tests in 100 and 1000 test kits for Aerobic Count, *E.coli*/coliform, Enterobacteriaceae, Yeast/Mold and Heterotrophic Plate Count.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