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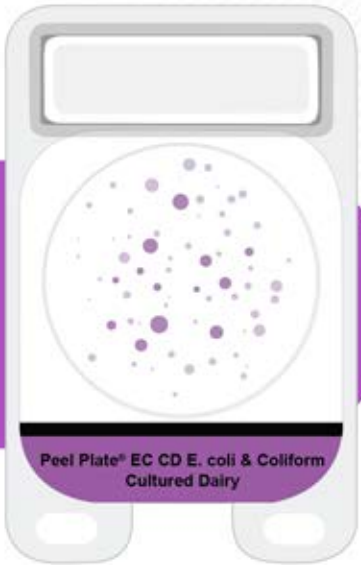
NOTHING WORKS LIKE A CHARM



OPERATOR'S MANUAL

Peel Plate® EC-CD

E. COLI & COLIFORM CULTURED DAIRY

WEBER SCIENTIFIC

FOR DETECTION AND ENUMERATION OF E.COLI AND COLIFORM BACTERIA IN CULTURED DAIRY PRODUCTS

Contents

Kit Information 4

- Introduction 4
- Kit Contents, Storage, and Testing Conditions 4
- Principle 5
- Applicability 5
- Precautions: 5

Sample Preparation 5

- Dairy (Liquid Cultured Drinks) 5
- Solid Dairy. 5
- Environmental Swab 6

Peel Plate EC-CD Procedure 6

Analysis of Results 7

Product Claim Limitations 8

Quality Control 8

Disposal 9

Technical Support 9

Order Information 9

Warranty 10

Kit Information

Introduction

Peel Plate® *E. coli* and Coliform Count for cultured dairy products test (EC-CD) detects and enumerates coliform bacteria in cultured dairy products like sour cream, cottage cheese, and cheeses that produce a red background on a Peel Plate EC test. The method is applicable for detection and distinguishing *E. coli* and coliform in cultured dairy products (with the exception of certain cheeses like feta and mozzarella that produce no background on a Peel Plate EC test and with the exception of some yogurts which may not differentiate the *E. coli* from coliform) when incubated at 32 ± 1 °C for 24 ± 2 hours. Green or blue colonies are interpreted as generic *E. coli* and red colonies are interpreted as coliform bacteria. The Peel Plate EC-CD tests are intended for microbiological laboratories, but may also be used by food quality stakeholders such as milk processors, engineers and water municipalities. 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-EC-CD-100K) contains 100 tests, 50 each in two desiccated foil bags containing a blue indicator desiccant. These contain a slightly different formulation than regular Peel Plate EC tests to facilitate coliform detection in cultured dairy products that produce reddish background in a regular Peel Plate EC test.

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 bag and remove the number of plates needed for analysis. Perform testing in a clean dry testing area at ambient temperature. **Tests held at room 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 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

Peel Plate EC-CD medium is based on EC broth medium to support and colorimetrically differentiate the growth of coliform in test samples. Peel Plate EC-CD tests contain the enzyme substrate salmon-gal (6-chloro-3-indolyl-B-D-galactopyranoside) used to detect ß-galactosidase produced by coliform and contains x-glucuronide (5-bromo-4-chloro-3-indolyl-B-D-glucuronide) used to detect ß-glucuronidase produced by *E. coli*. Additionally the EC-CD test has selective agents such as bisulfite to reduce red background seen with cultured dairy products tested on a regular EC test. Peel Plate EC-CD tests also contain gelling and wicking agents which absorb and diffuse the sample.

Applicability

Peel Plate EC-CD test has been validated for detection of *E. coli* and coliform in cultured dairy (sour cream, cottage cheese, cheeses) and found not significantly different from reference method Violet Red Bile Agar (VRBA) with colonies confirmed with Brilliant Green Lactose Bile (BGLB) broth. Some ingredients in some yogurts interfere with the *E. coli* blue color development so it is recommended to test yogurt for coliform only. Some cheeses like mozzarella and feta do not produce a background on the regular Peel Plate EC formulation and those cheese types should use the Peel Plate EC test. 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 EC-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 done to achieve a countable range on the plate.

Solid Dairy

- Add 11 g of solid dairy (sour cream, cottage cheese, cheeses, etc.) to 99 mL of

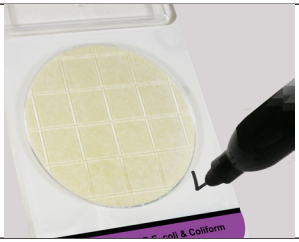
microbiologically suitable dilution blanks to reach countable range (1 to 154 CFU/mL). Mix/homogenize and let any undissolved solids settle (no more than 3 minutes).

- For dried powders (e.g. Whey), reconstitute 1:10 with diluent and let any undissolved solids settle (no more than 3 minutes).

Environmental Swab

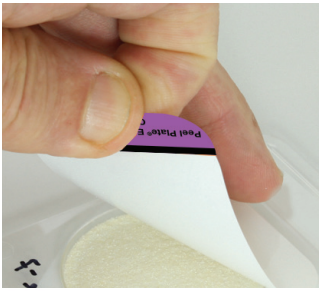
- Refer to Peel Plate Sample Preparation Addendum.

Peel Plate EC-CD Procedure



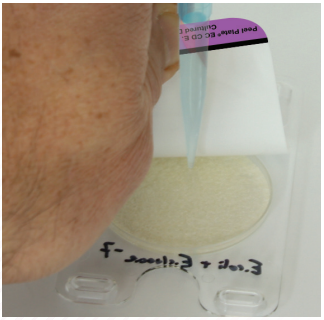
Step 1

- 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 EC-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.

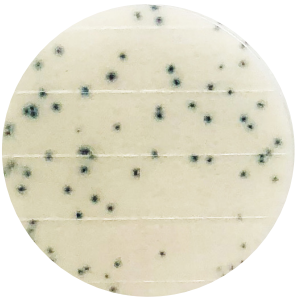
Step 4

- Sample will diffuse towards the edges of plate. For viscous samples 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.
- Allow plate to sit 30 seconds before moving.

Step 5

- Incubate plates in the dark with clear side up, as shown.
  - Incubate at **32° ± 1 °C for 24 ± 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 red spot represents one CFU coliform and each blue and green spot represents one CFU *E. coli*. 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/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 cm^2, 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.
- Any background should be consistent within a matrix and colonies should be easily distinguishable from any background in 24 hours.
- 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 EC-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

- Peel Plate tests have been evaluated in claimed foods, but have not been evaluated with all possible food products, food processes, testing protocols, or with all possible microorganism strains.

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.

Order Information

Description	Quantity	Kit Code
Peel Plate for *E.coli*/coliform in Cultured Dairy	50	4150-42

Peel Plate tests are also available in 5 mL sample volume tests in 100 and 1000 test kits for Aerobic Count, 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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