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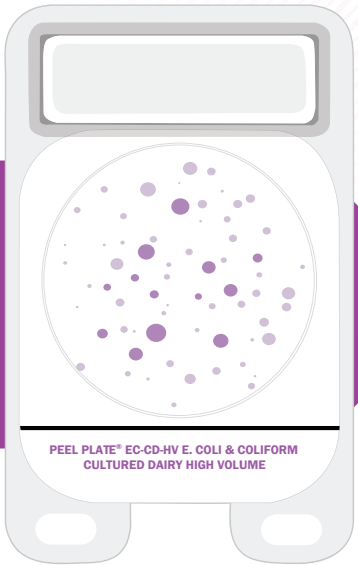

OPERATOR'S MANUAL

Peel Plate® EC-CD-HV

E. COLI & COLIFORM CULTURED DAIRY HIGH VOLUME

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

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Kit Information

Introduction

Peel Plate® *E. coli* and Coliform Count Cultured Dairy High Volume (EC-CD-HV) Test detects and enumerates *E. coli*/coliform bacteria in 5 mL sample volumes. The method is applicable for detection of total coliform in cultured dairy products like, cottage cheese, sour cream, kefir, cheeses, and other fermented dairy products with active cultures with the exception of yogurts, when incubated at 32° ± 1 °C for 24 ± 2 hours. Blue colonies are interpreted as generic *E. coli* and red colonies are interpreted as coliform bacteria. A 5 mL sample of a 1:10 diluted homogenate is added to each of 2 HV plates and incubated in the dark at 32° ± 1 °C. The Peel Plate EC-CD-HV test is intended for microbiological laboratories, but may also be used by food quality stakeholders such as farmers, milk processors, engineers and water municipalities. The method limit of detection is 1 or greater coliform or *E. coli* colony forming units per 5 milliliter (CFU/5 mL) of test sample. The quantitative range for coliform is defined as 1 to 154 CFU/plate.

Kit Contents, Storage, and Testing Conditions

A 100 test kit (Kit Code: PP-EC-CD-HV-100K) contains 4 desiccated foil bags, each containing 25 Peel Plate EC-CD-HV tests (formulation on 75 mm plates).

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 or 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 EC-CD-HV medium is based on conventional coliform selective medium to support and colorimetrically differentiate *E. coli*/coliform in test samples. The Peel Plate EC-CD-HV test

contains the enzyme substrates salmon-gal (6-chloro-3-indolyl-B-D-galactopyranoside) used to detect ß-galactosidase enzyme produced by coliform, and x-glucuronide (5-bromo-4-chloro-3-indolyl-B-D-glucuronide) used to detect ß-glucuronidase produced by *E. coli*. Additionally the EC-CD-HV formulation includes pH adjustment and background reduction agents, such as bisulfite that are not incorporated in the EC HV test. The Peel Plate EC-CD-HV test also contains gelling and wicking agents which absorb and self-diffuse the sample.

Applicability

The Peel Plate EC-CD-HV test is applicable in liquid cultured dairy (e.g. kefir), and solid cultured dairy (e.g. sour cream, cottage cheese, and cheeses). The Coliform Count Peel Plate for cultured Dairy is recommended for yogurt due to select ingredients that interfere with the *E.coli* indicator.

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-HV medium.

Sample Preparation

Dairy (Liquid Cultured Drinks)

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

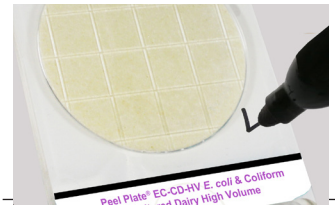
Solid Dairy

- Add 11 g of solid dairy (cottage cheese, sour cream, 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 EC-CD-HV Procedure



Step 1

- Label plate on clear side using a marker. Do not mark or label the uplifted 75 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-HV media.

Step 3

- Rapidly dispense 5.0 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.

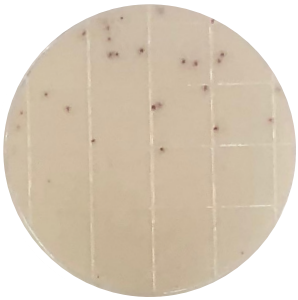
Step 4

- 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 12 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 coliform CFU and each blue spot represents one *E. coli* CFU. The sum of spots is reported as the total coliform CFU/5 mL of the diluted sample.
- Sum the results of two 5 mL plates of 1:10 dilution to calculate CFU/mL or CFU/gram of original sample.
- In case of spreading bacteria, score one CFU for each defined separated spot. Blended or spreading colonies are scored as a single CFU.
- Counts of 1 to 154 CFU/plate are considered in countable range, while counts outside that range are considered estimates. Samples with results outside of countable range (>154 CFU/plate) can be diluted and retested.
- Cultured samples containing active LAB, e.g. 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.

- 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.

Product Claim Limitation

- 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 5.0 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 5.0 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 5.0 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

The Peel Plate EC-CD-HV test is supplied in 25 (Kit code: PP-EC-CD-HV-25K) and 100 (Kit code: PP-EC-CD-HV-100K) test kits.

Peel Plate tests are also available in 1 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 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.

OM-763-002

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OM-763-002 May-2019