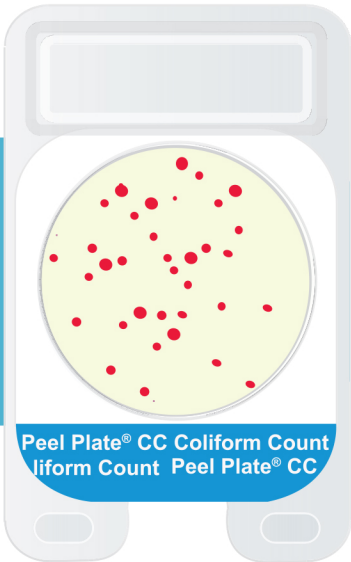




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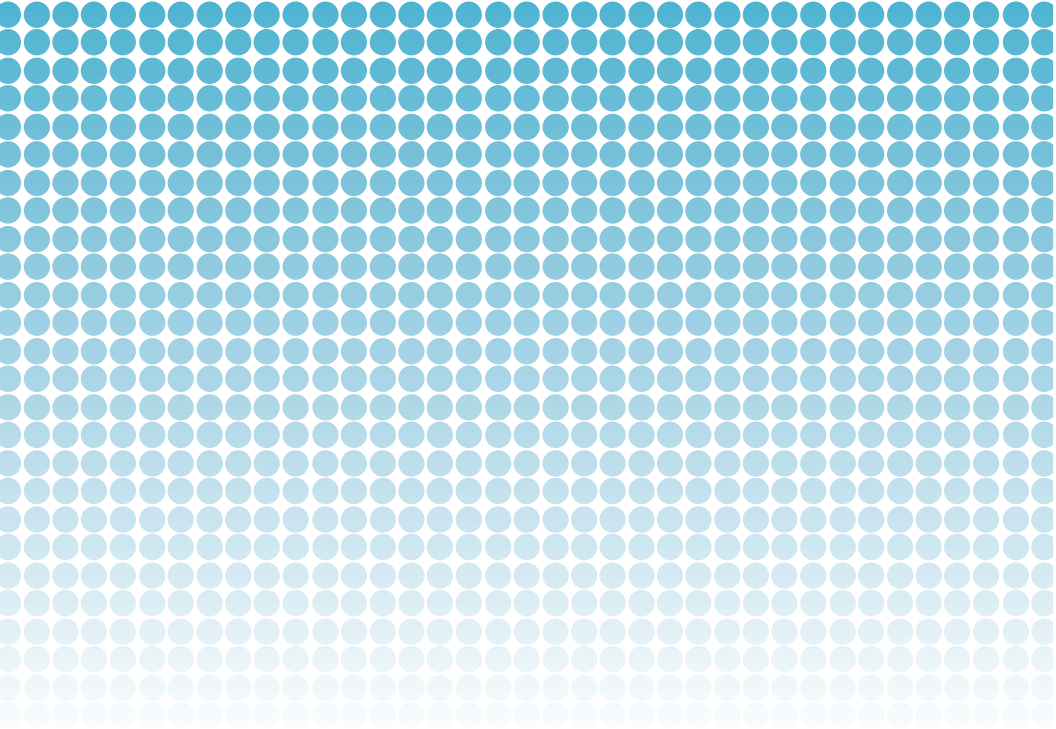


OPERATOR'S MANUAL



PeelPlate[®] CC
COLIFORM

FOR DETECTION AND ENUMERATION OF TOTAL COLIFORM BACTERIA



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Kit Information

Introduction

Peel Plate® CC (Total Coliform) tests detect and enumerate total coliform bacteria. The method is applicable for determination of total coliform in dairy products (with the exception of certain hard cheeses) when incubated at 32 ± 1 °C and in food dilutions, surface sponge and water samples when incubated at 35 ± 1 °C for 24 hours. To test hard cheeses use the Peel Plate CC-C test. Sample or sample dilution is added and incubated for 24 ± 2 hours, at 32 ± 1 °C or 35 ± 1 °C depending on food type tested. Peel Plate CC 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/test.

Kit Contents, Storage, and Testing Conditions

A test kit (item code PP-CC-100K) contains 100 tests, 50 each in two desiccated foil bags containing a blue indicator desiccant. A kit for yogurt and cultured dairy product testing (item code PP-CCS03-100K) contains a sodium bisulfite concentrate for sample preparation. A kit specifically for hard cheeses (item code PP-CC-C-100K) contains a slightly different formulation to facilitate coliform detection.

Kits are not required to be shipped refrigerated.

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

Open bag and perform testing in a clean dry testing area at ambient temperature. Remove number of plates need for analysis. **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 test will discolor yellow. Do not use discolored tests or tests from bags with a pink/white desiccant indicator.

Principle

Peel Plate CC medium is based on EC broth medium to support and colorimetrically differentiate the growth of coliform in test samples at 35 ± 1 °C. Peel Plate CC tests contains the enzyme substrate salmon-gal (6-chloro-3-indolyl-B-D-galactopyranoside) used to detect β -galactosidase produced by coliform. Peel Plate CC tests also contains gelling and wicking agents which absorb and diffuse the sample.

Applicability

Peel Plate CC test is very similar to the Peel Plate EC test except that the enzyme indicator for generic *E. coli*, X-gluc, has been removed. Peel Plate EC has been certified by the AOAC Research Institute as Performance Tested Method #061501. This test kit's performance was reviewed by AOAC-RI and was found to perform to the manufacturer's specifications. The method has been validated for detection of total coliform in liquid dairy (raw and pasteurized milk, skim, chocolate, cream), solid dairy (reconstituted non-fat dry milk, lactose reduced, vanilla ice cream, sour cream, condensed/evaporated, other flavored, condensed whey), and cultured dairy homogenized in sodium bisulfite diluent before testing (cottage cheese, yogurt) and found not significantly different from reference method Violet Red Bile Agar (VRBA) with colonies confirmed with Brilliant Green Lactose Bile (BGLB) broth. Some cheddar and other hard cheeses like Parmesan, Romano, Swiss, Provolone use a separately formulated method, Peel Plate CC-C. At 35 ± 1 °C Peel Plate EC test has been validated in ground meat, environmental surface sponges, carcass rinse and sponges, and 0.45 μ m filtered drinking and vegetable/fruit process water, and found not significantly different from FDA-BAM or USDA-FSIS or EPA official reference methods. Samples should be 10-fold serially diluted into the countable range of 1 to 154 CFU/mL.

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 blowing air.
- Avoid hand contact with test samples and Peel Plate CC medium.
- After plating, re-seal adhesive cover so that it lays flat with no wrinkles to avoid drying out the rehydrated medium during incubation.

Sample Preparation

Dairy	<ul style="list-style-type: none"> White milk dairy samples (raw milk and pasteurized whole, lower fat %, and skim) may be tested directly or serially diluted to a countable range (1 to 154 CFU/mL). <ul style="list-style-type: none"> To serially dilute, add 11 mL into 99 mL microbiologically suitable dilution blanks. Other automated dilution pipets and dilution schemes are acceptable. Neat chocolate milk should be diluted 1 part to 1 part dilution buffer, and 1 mL each plated onto two plates in duplicate (total of 4 plates per sample). Chocolate milk may also be serially diluted into a countable range (1 to 154 CFU/mL).
Solid Dairy	<ul style="list-style-type: none"> Add 11 g of solid dairy (ice cream, sour cream, heavy cream, etc.) to 99 mL of microbiologically suitable dilution blanks to reach countable range (1 to 154 CFU/mL). For fermented solid dairy (cottage cheese, yogurt, condensed whey, etc.) containing active lactic acid bacteria (LAB) culture. <ul style="list-style-type: none"> Add 11 g product to 99 mL dilution blank heated to 40 to 45 °C and mix/homogenize. Add 1 mL of sterile sodium bisulfite solution (item BUF-NAS03 supplied with PP-CCS03-100K kit) to the mix/homogenate and mix well. <ul style="list-style-type: none"> Alternatively dissolve 0.2 g sodium bisulfite powder in 99 mL dilution blank, add 11 g product, and mix/homogenize. For milk powders and evaporated/condensed, reconstitute with water to normal milk solid content and let any undissolved solids settle. Test liquid fraction as Dairy. Certain cheeses that produce a red background on Peel Plate CC should use an alternative formulated Peel Plate CC-C.
Ground Meat	<ul style="list-style-type: none"> Add 50 g of ground meat to 450 mL of microbiologically suitable dilution blank, stomacher blend, and let settle to extract sample. Continue to dilute 10 mL of prior dilution in 90 mL of dilution blank to reach countable range (1 to 154 CFU/mL).
Liquid Egg	<ul style="list-style-type: none"> Add 100 g of liquid eggs to 900 mL of microbiologically suitable dilution blank, stomacher blend, and let settle to extract sample. Continue to dilute 10 mL of prior dilution in 90 mL of dilution blank to reach countable range (1 to 154 CFU/mL).
Carcass Swab	<ul style="list-style-type: none"> Refer to Peel Plate Sample Preparation Addendum.
Environmental Swab	<ul style="list-style-type: none"> Refer to Peel Plate Sample Preparation Addendum.

Peel Plate CC Test Procedure



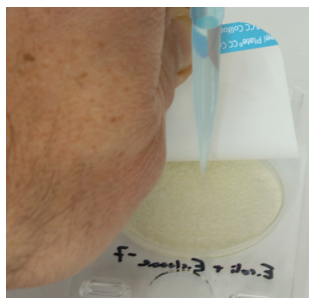
Step 1

- Label plate on clear side using marker or bar code strip. Do not mark or label the uplifted 47 mm circular area.



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.



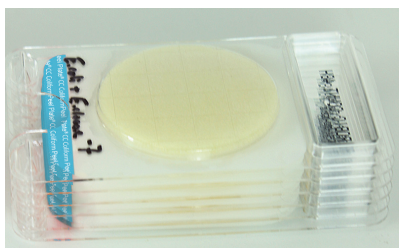
Step 3

- 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 32 ± 1 °C for 24 ± 2 hours for milk and dairy products. Incubate an additional 24 hours when testing yogurt.
- Incubate at 35 ± 1 °C for 24 ± 2 hours for water, environmental, and meat samples.
- Plates can stack by aligning the feet and back rectangular platform. Stacking will not affect plate heat transfer.

Analysis of Results



- At the end of incubation, observe plates for colonies through the clear side of the Peel Plate CC test. Each red spot represents one CFU coliform. 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 the case of neat chocolate milk, add the sum of the two plates of the 1 to 2 diluted product for a CFU/mL of neat product.
- 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/test 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^2 , and multiplying by 17.4 for estimated colonies per plate (eCFU/plate).

- Cultured samples containing active LAB, e.g. yogurts, may present a reddish background and requires 24 hour additional incubation. Count distinct darker red colonies as coliform. Cheeses that produce a strong red background need to use an alternative formulated Peel Plate CC-C test.

Optional Colony Counter:

- Insert completed test into the Colony Counter. Identify the plate as Peel Plate CC.
- 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.

Interpretation of Results

- In inclusivity and exclusivity studies with Peel Plate EC test, the sister test to Peel Plate CC test, 57 of 58 coliform inclusivity isolates were correctly detected as coliform, including all 17 *E. coli* strains.
- 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 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:** Spike a sample with known coliform culture. Dilute sample to countable range of 1 to 154 CFU/mL and test 1.0 mL to verify detection after incubation.

Disposal

Microbiological cultures and reagents should be collected into biohazard bags and autoclaved. 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 CC	100	PP-CC-100K
	1000	PP-CC-1000K
Peel Plate for Coliform in Hard Cheeses	100	PP-CC-C-100K
Peel Plate CC Tests including sodium bisulfite concentrate	100	PP-CCS03-100K
Sodium bisulfite Concentrate	1	BUF-NAS03

Peel Plate tests for *E. coli* and coliforms, aerobic bacteria, *Enterobacteriaceae* 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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