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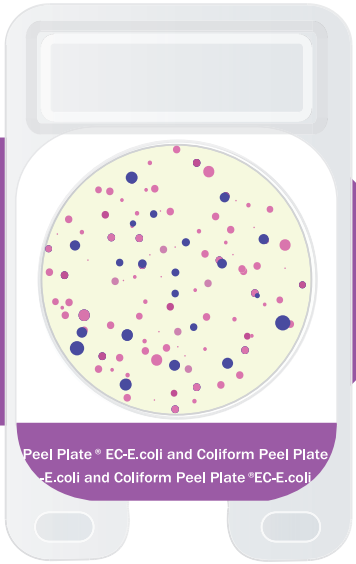


# OPERATOR'S MANUAL

## PeelPlate® EC

### E.COLI AND COLIFORM

FOR DETECTION AND ENUMERATION OF TOTAL COLIFORM BACTERIA INCLUDING *E.COLI*

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# Kit Information

## Introduction

Peel Plate® EC (*E. coli* and Coliform) tests detect and enumerate total coliform bacteria including *E. coli* . The method is applicable for determination of total coliform, sum of coliform and *E. coli* , in food dilutions, surface sponge and water samples based on colony color when incubated at 35 ± 1 °C. The method will distinguish *E. coli* from other coliform in dairy products when incubated at 32 ± 1 °C. Sample or sample dilution is added and incubated for 24 ± 2 hours, at 35 ± 1 °C or 32 ± 1 °C depending on food type tested and microbial detection objectives. Peel Plate EC tests are intended for microbiological laboratories, but may also be used by food quality stakeholders such as farmers, milk processors, 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 including *E. coli* is defined as 1 to 154 CFU/test.

## Kit Contents, Storage, and Testing Conditions

A test kit (item code PP-EC-100K) contains 100 tests, 50 each in two desiccated foil bags containing a blue indicator desiccant. A kit for cultured dairy product testing (item code PP-EC-CD-100K) is available for applicable products.

Kits are not required to be shipped refrigerated.

**Store kits in foil bag refrigerated*** or at room temperature, ambient temperature 0 to 25° C, until expiration date.

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.

*** Refrigeration is defined as 0 to 4.5 °C and is required for US Certified Labs**

## Principle

Peel Plate EC medium is based on EC broth medium to support and colorimetrically differentiate the growth of coliform and *E. coli* in test samples at 35 ± 1 °C. Peel Plate EC tests contains the enzyme substrates salmon-gal (6-chloro-3-indolyl-B-D-galactopyranoside) used to detect ß-galactosidase produced by coliform and x-glucuronide (5-bromo-4-chloro-3-indolyl-B-D-glucuronide) used to detect ß-glucuronidase produced by *E. coli* when incubated at 35±1 °C. Peel Plate EC tests also contain gelling and wicking agents which absorb and diffuse the sample.

## Applicability

Peel Plate EC test 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. At 35±1 °C Peel Plate EC test has been validated to distinguish coliform from *E. coli* in ground meat, environmental surface sponges, carcass rinse and sponges, and 0.45 µm filtered drinking and vegetable/fruit process water, and found not significantly different from FDA-BAM or USDA-FSIS or EPA official reference methods. At 32±1 °C, 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, condensed/evaporated, other flavored) 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/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 EC 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 and Other Liquid Dairy Samples** | • 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).<br>› To serially dilute, add 11 mL into 99 mL microbiologically suitable dilution blanks. Other automated dilution pipets and dilution schemes are acceptable.<br>• Dilute chocolate milk samples 1 part sample to 1 part dilution buffer and plate in duplicate. Chocolate milk may also be serially diluted into a countable range (1 to 154 CFU/mL). |
| **Solid Dairy** | • Add 11 g of solid dairy (ice cream, heavy cream, etc.) to 99 mL of microbiologically suitable dilution blanks to reach countable range (1 to 154 CFU/mL).<br>• For fermented solid dairy (cottage cheese, sour cream, yogurt, shredded cheese, condensed whey, etc.) containing active lactic acid bacteria (LAB) culture, use PP-EC-CD test.<br>• 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. |
| **Ground Meat** | • Add 50 g of ground meat to 450 mL of microbiologically suitable dilution blank, stomacher blend, and let settle to extract sample.<br>• 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** | • Add 100 g of liquid eggs to 900 mL of microbiologically suitable dilution blank, stomacher blend, and let settle to extract sample.<br>• 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** | • **Refer to Peel Plate Sample Preparation Addendum.** |
| **Environmental Swab** | • **Refer to Peel Plate Sample Preparation Addendum.** |
| **Bottled Water and Filtered Water Samples** | • **Refer to Peel Plate EC Water Filtration Procedure.** |

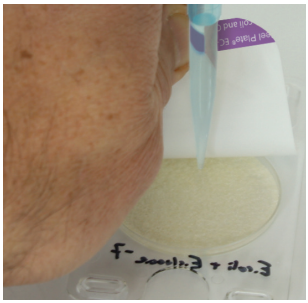
# Peel Plate EC Test Procedure

Step 1

- For ease of opening, use plates at room temperature.
- 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.

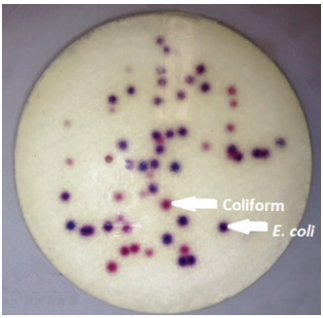
Step 4

- Sample will diffuse to the edges of the disc. 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 with clear side up, as shown.
- Incubate at 35 ± 1 °C for 24 ± 2 hours for all non-dairy samples, including for water, liquid egg, environmental swabs, and meat
- Incubate at 32 ± 1 °C for 24 ± 2 hours for milk and dairy products.
- Plates can stack up to 20 high by aligning the feet and back rectangular platform. Stacking will not affect plate heat transfer.

## Analysis of Results




**Coliform (red) colonies = 20**

***E. coli* (blue/black) = 34**

**Total coliform = 54 = 20 + 34**

- At the end of incubation, observe plates for colonies through the clear side of the Peel Plate EC test. Each colored spot, blue and red, represents one CFU. The sum of spots is reported as the total coliform CFU/mL of the diluted sample.
- Multiply CFU/mL by dilution to calculate CFU/(mL or g) of original sample. In the case of chocolate milk, add the sum of the two plates of the 1 to 1 diluted product for a CFU/mL of neat product.
- *E. coli* is a member of the coliform group and can be differentiated colorimetrically by dark blue, purple, or greenish color at 35 ± 1 °C (see Interpretation of Results). Coliform bacteria will appear red (salmon) in color (see Interpretation of Results). The count of red colonies is reported as coliform and the count of blue/purple/green/black is reported as *E. coli* . Sum of colonies is total coliform (see Interpretation of Results).
- 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 and blue/purple colonies as coliform.

### Optional Peel Plate Colony Counter:

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

## Interpretation of Results

- In 2015 inclusivity studies, 57 of 57 coliform inclusivity isolates were correctly detected, including all 17 *E. coli* strains. Escherichia blattae, ATCC 29907 was not detected but is no longer classified as Escherichia but rather Shimwellia, and is no longer considered a coliform. Six of the 17 *E. coli* isolates produced red colonies instead of the typical blue/purple/green colonies. This may be because they are weak producers or do not produce glucuronidase enzyme that is produced by majority of generic *E. coli* strains. In 2021, 63 of 63 coliform isolates were correctly detected. Nine of the 30 *E. coli* isolates produce purple/red colonies instead of blue making it difficult to distinguish from coliform. In both studies shiga type *E. coli* produced red colonies as they are known not to produce the glucuronidase enzyme that causes blue/purple. In 2015, of the 36 exclusivity strains evaluated, 32 were correctly excluded. In 2021, of the 40 exclusivity strains evaluated, 36 were correctly excluded. The strains that were detected as coliform were, Shigella sonnei, ATCC 9290, Serratia marcescens ATCC 8100, Serratia marcescens ATCC 13880, and Shigella sonnei ATCC 25931. Incubation of dairy products at 32 ± 1 °C does not reliably induce β-glucuronidase enzyme in all strains of *E. coli* and they might produce red color only. Absence of blue colonies should not be interpreted as absence of *E. coli*.
- Peel Plate EC 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.
- Bottled water has been evaluated, but the method has not been evaluated for municipal water testing in compliance with EPA Total Coliform Rule.

## 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 or a combination of coliform and *E. coli* 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 EC | 100 | PP-EC-100K |
| | 1000 | PP-EC-1000K |

Peel Plate tests for high volume coliforms, aerobic bacteria, total coliform, enterobacteriaceae, yeast and mold, 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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