

## Operator's Manual:



### Kit Information

#### Introduction

Peel Plate<sup>®</sup> 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 dairy products when incubated at 32±1 °C. The method will distinguish *E. coli* from other coliform in food dilutions, surface sponge and water samples based on colony color when incubated at 35±1 °C. 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 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-ECSO3-100K) contains a sodium bisulfite concentrate for sample preparation.

**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 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 β-galactosidase produced by coliform and x-glucuronide (5-bromo-4-chloro-3-indolyl-B-D-glucuronide) used to detect β-glucuronidase produced by *E. coli* when incubated at 35±1 °C. Peel Plate EC tests also contains 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. 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, shredded cheese) and found not significantly different from reference method Violet Red Bile Agar (VRBA) with colonies confirmed with Brilliant Green Lactose Bile (BGLB) broth. 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. Samples should be 10-fold serially diluted into the countable range of 1 to 154 CFU/mL.

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© 2016 Charm Sciences Inc. The Charm and Peel Plate names are registered trademarks of Charm Sciences Inc. See www.charm.com/en/us-patents for a list of U.S. issued patents and pending, published U.S. and PCT applications.



## 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

- 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).
  - 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

- 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, shredded cheese, condensed whey, etc.) containing active lactic acid bacteria (LAB) culture
  - Add 11 g product to 99ml dilution blank and mix/homogenize
  - Add 1 mL of sterile sodium bisulfite solution (item BUF-NAS03 supplied with PP-ECS03-100K kit) to the mix/homogenate and mix well.
    - 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.

### Ground Meats

- 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 Eggs

- 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

- 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**.

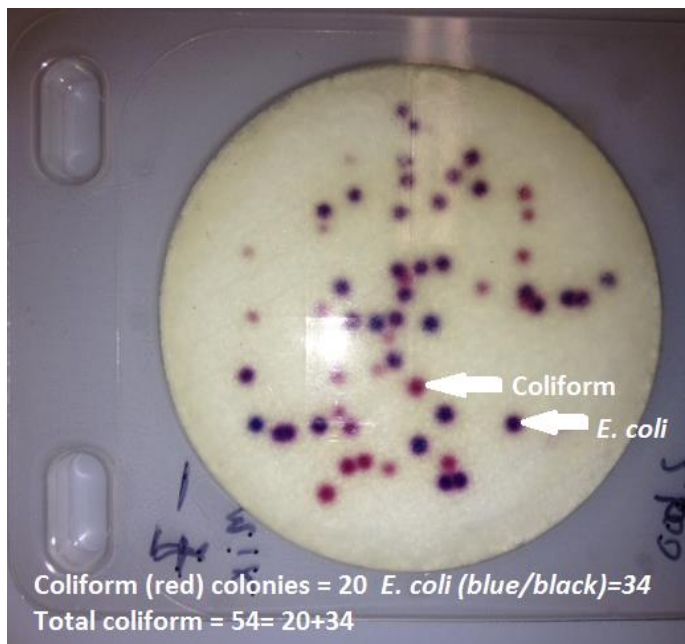
# Peel Plate EC Procedure

			<p><b>Step 1</b></p> <ul style="list-style-type: none"><li>Label plate on clear side using marker or bar code strip. Do not mark or label the uplifted 47mm circular area.</li></ul>
			<p><b>Step 2</b></p> <ul style="list-style-type: none"><li>Invert and place test onto a level surface. Apply pressure with fingers to the back platform as shown and lift tab.</li><li>Pull the adhesive cover exposing the culture disc. Leave cover adhered to back of plate.</li></ul>
			<p><b>Step 3</b></p> <ul style="list-style-type: none"><li>While holding cover up, and keeping plate flat on surface, <b>vertically dispense 1.0 mL onto the center of disc.</b> Expel in 2 to 3 seconds while 1 to 2 cm from surface.</li></ul>
			<p><b>Step 4</b></p> <ul style="list-style-type: none"><li>Sample will diffuse to the edges of the disc.</li><li>Re-seal the adhesive cover without wrinkling. Press around edges of plate to ensure proper seal.</li></ul>



- Step 5**
- Incubate plates with clear side up, as shown.
    - Incubate at  $32 \pm 1^\circ\text{C}$  for  $24 \pm 2$  hours for milk and dairy products. Incubate an additional 24 hours when testing yogurt.
    - Incubate at  $35 \pm 1^\circ\text{C}$  for  $24 \pm 2$  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 Peel Plate EC Results



- 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 neat 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 is differentiated colorimetrically by dark blue, purple, or greenish color at  $35 \pm 1^\circ\text{C}^*$ . Coliform bacteria will appear red (salmon) in color\*. The count of red colonies is reported as coliform and the count of blue/purple/black is reported as *E. coli*. Sum of colonies is total coliform\*.
- 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<sup>2</sup>, and multiplying by 17.4 for estimated colonies per plate (eCFU/plate).
- Cultured samples containing active LAB, i.e. 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 Reader:

- Insert completed test into the Peel Plate test Reader. Identify the plate as Peel Plate EC.
- Enter sample identity or verify that bar code 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 Reader instructions.

## \*Interpretation of Results

- In inclusivity and exclusivity studies, 57 of 58 coliform inclusivity isolates were correctly detected, including all 17 *E. coli* strains. The coliform strain not detected was *Escherichia blattae*, ATCC 29907. 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. Two of those strains, O157:H7 and O145, are shiga type *E. coli* known not to produce the enzyme. Of the 32 exclusivity strains evaluated, 31 were correctly excluded. The strain that was detected as coliform was, *Shigella sonnei*, ATCC 9290. 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.

## Order Information

Description	Quantity	Kit Code
Peel Plate EC Tests	100	PP-EC-100K
	1000	PP-EC-1000K
Peel Plate EC Tests including sodium bisulfite concentrate	100	PP-ECSO3-100K
Sodium bisulfite Concentrate	1	BUF-NASO3
Peel Plate EC High Volume 5mL including BUF-NASO3	100	PP-ECHVS-100K

Peel Plate tests for *E. coli* and coliforms, aerobic bacteria, and heterotrophic bacteria are also available. Visit Charm Sciences' website at [www.charm.com](http://www.charm.com) to learn more.

## Technical Support

For questions contact a local representative or Charm Sciences at +1.978.687.9200 or [support@charm.com](mailto:support@charm.com).

## Warranty

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