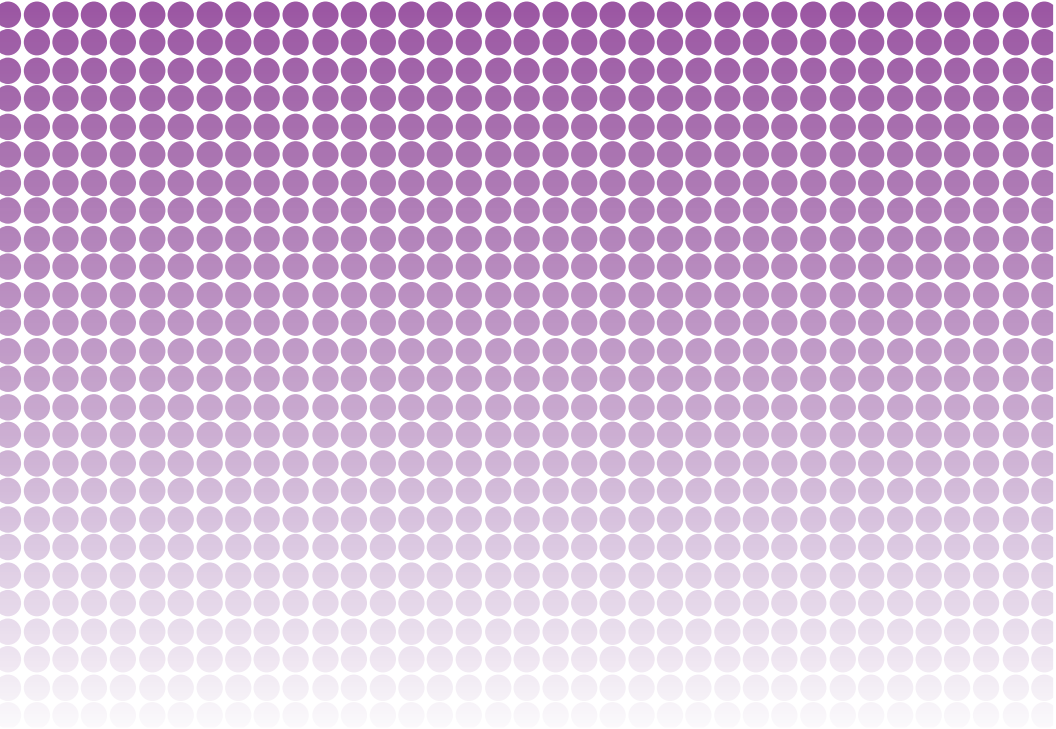


OPERATOR'S MANUAL FOR FILTERED SAMPLES



FOR DETECTION AND ENUMERATION OF TOTAL COLIFORM AND *E. COLI*
BACTERIA USING FILTERED SAMPLES



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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 determining coliform and *E. coli* in water samples and determining potability of water in 100 mL filtered samples. A mixed-cellulose 0.45 µm membrane filter is employed for filtration. After sample filtration, the filter is added to a rehydrated Peel Plate EC test and incubated at 35°C ± 1 for 24 hours. The method principle of detection is described in the Peel Plate EC test operator's manual OM-600. The method is intended for water quality stakeholders such as farmers, field environmentalists, and water municipalities. The method limit of detection is 1 or greater colony forming units per 100 milliliters (CFU/100 mL) of the test sample.

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. Supplied separately are 0.45 µm mixed-cellulose filters for sample collection and pipet tips or disposable pipet bulbs for test hydration.

Kits are not required to be shipped refrigerated.

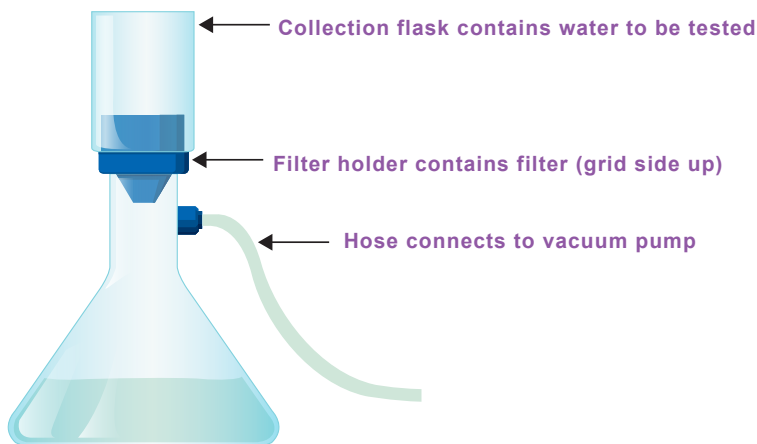
Store kits in foil bag refrigerated* or at controlled room temperature (0 to 25°C), until expiration date.

Open the bag and remove the number of plates needed for analysis. Reseal the bag using the zip closure to store unused tests. Perform testing in a clean dry testing area at ambient temperature. Tests held at room temperature for 1 hour prior to use will open more easily. 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**

Equipment Needed

Water sample filtration apparatus including vacuum, collection flask, and filter holder with stopper to flask.



Applicability

The 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 $35^{\circ}\text{C} \pm 1$, the Peel Plate EC test with use of filtration has been validated to distinguish coliform from generic *E. coli* in 0.45 μm filtered bottled water, irrigation water and vegetable/fruit process waters. The method does not distinguish pathogenic shiga toxin producing *E. coli* as these are known to lack the color distinguishing enzyme. The method was 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. With surface waters containing high heterotrophic bacteria, addition of cefsulodin at 5 $\mu\text{g/mL}$ is recommended for plate rehydration.

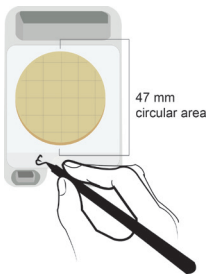
Precautions

Observe Good Laboratory Practices for microbial testing. Avoid specimen contamination.

- Perform tests with clean and gloved hands, assuming potential pathogenic bacteria.
- Test on a level surface, in a clean area and free of dust and drafts.
- If taking samples from spigot, run water for 30 seconds to flush water line before sampling.

- Avoid hand contact with test samples, water containers, 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.

Procedure



Step 1

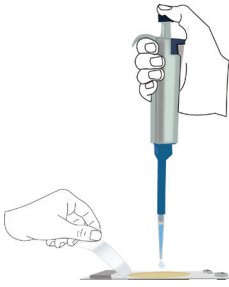
- Place filter into filtration apparatus, grid side up. See diagram in **Equipment Needed**.
- Pour sample into filter unit.
- Apply partial vacuum to draw water through the filter membrane.

Step 2

- 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 3

- Place Peel Plate test onto a level surface. Lift tab and apply pressure with fingers to the back platform as shown.
- Pull the adhesive cover back enough to completely expose the culture disk. To lessen potential contamination, do not entirely remove the cover.



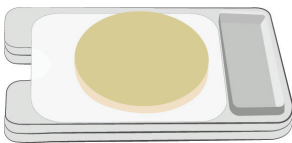
Step 4

- Use pipet to vertically dispense 1.5 mL of sterile deionized water^A or preferred sterile broth to the center of the exposed Peel Plate disc.^A Expel pipet contents evenly, within 1 to 3 seconds.
- Liquid will wick to the edges as the disc rehydrates.



Step 5

- Use sterile tweezers to aseptically remove the filter from filter apparatus.
- Place the filter, grid side up, centered onto the wetted Peel Plate disc. Roll the filter into position to avoid trapping air.
- Lightly press edges of the filter with tweezers to adhere filter to Peel Plate disc.
- Re-apply the adhesive cover. Press around the edges of the plate to ensure proper seal.



Step 6

- Incubate the plates with clear side up.
- Incubate $35 \pm 1^\circ\text{C}$ for 24 ± 2 hours.
- Plates can stack up to 20 high by aligning the feet and back rectangular platform. Stacking will not affect plate heat transfer.

^A**NOTE**- for surface waters use 5 $\mu\text{g}/\text{mL}$ cefsulodin solution to reduce interfering pigmented bacterial growth

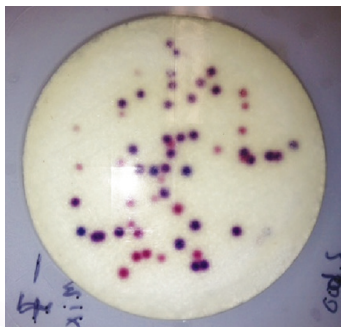
Analysis of Results

At the end of the incubation period, observe plates for colonies viewed from both the non-adhesive side and the adhesive side after removal of adhesive cover.

- Each colored spot, red and blue/green/purple, represents one coliform colony forming unit (CFU).
- The sum of all spots is reported as the total coliform CFU/filtered volume. The sum of blue/green/purple colonies are *E. coli* CFU/filtered volume.* Salmon/red colored colonies are coliform CFU/filtered volume.
- Surface waters may present cultured bacteria that are non-coliform with pink pigmentation. The light pink pigment is distinguishable from the dark red coliform colony and therefore the pink should not be counted. Rehydration of the Peel Plate test with an antibiotic, such as 5 µg/mL cefsulodin, may reduce interference from non-coliform in surface waters.
- Counts of 1 to 154 CFU/filter are considered countable, while counts outside that range are considered estimates. Samples with results outside the countable range should 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 cm², and multiplying by 17.4 for estimated colonies per plate (eCFU/plate). This is the estimate of counts per filter. .

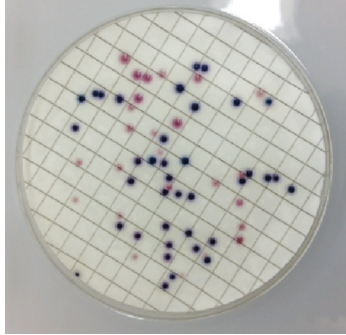
Colonies as viewed through Peel Plate underside

- *E. coli* are fecal coliform and are distinguished by their blue, or purple, or greenish color. Other coliform bacteria will be red (salmon) color.



Colonies as viewed on filter with adhesive cover removed

- In case of spreading bacteria, score one CFU for each dark spot and separated color circle. Blended colonies are scored as a single CFU.



*Avoid misinterpretation of results

- *E. coli* 0157 or *E. coli* 0157 H7 and some other verotoxigenic *E. coli* strains do not produce the enzyme β -glucuronidase and are detected as a coliform only (red colony) at $35\text{ }^{\circ}\text{C} \pm 1$.
- In the presence of red coliform, absence of blue colonies should not be necessarily be interpreted as absence of *E. coli*.
- Peel Plate EC plates have been evaluated in claimed foods and water, but have not been evaluated with all possible food and water products, processes, testing protocols or with all possible microorganism cultures.
- 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.

- **Negative Control:** Test 1 mL of sterile water on a Peel Plate test to verify no detectable bacteria on the Peel Plate test after incubation.
- **Filter Negative Control:** Prepare Negative Control by autoclaving 100 mL water at 121 °C for 15 minutes. Cool, then filter sample and test. Verify no detectable bacteria on the filter.
- **Positive Control:** Spike a 100 mL water sample with known coliform culture to make a Positive Control.

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.

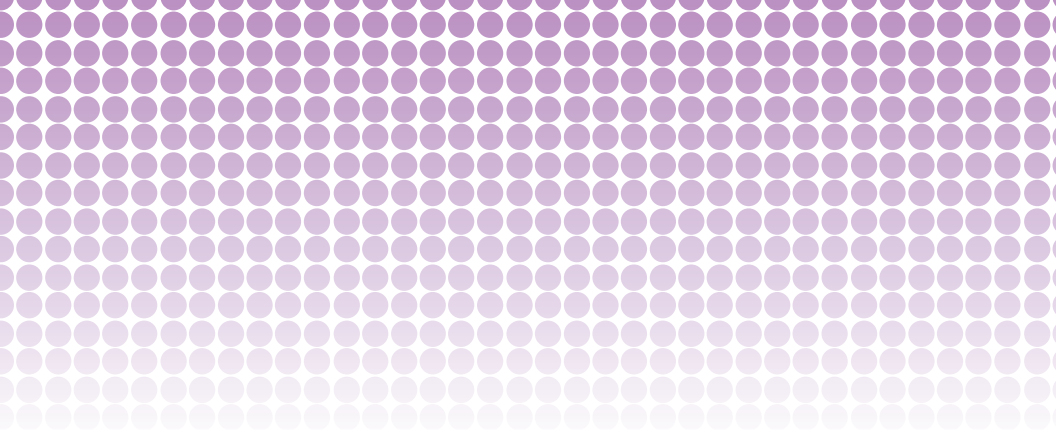
Order Information

Description	Quantity	Kit Code
Peel Plate EC	100	PP-EC-100K
	1000	PP-EC-1000K
Peel Plate EC High Volume 5 mL	100	PP-ECHV-100K

Peel Plate tests for *E. coli* and coliforms, total coliform, enterobacteriaceae, aerobic bacteria, 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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