

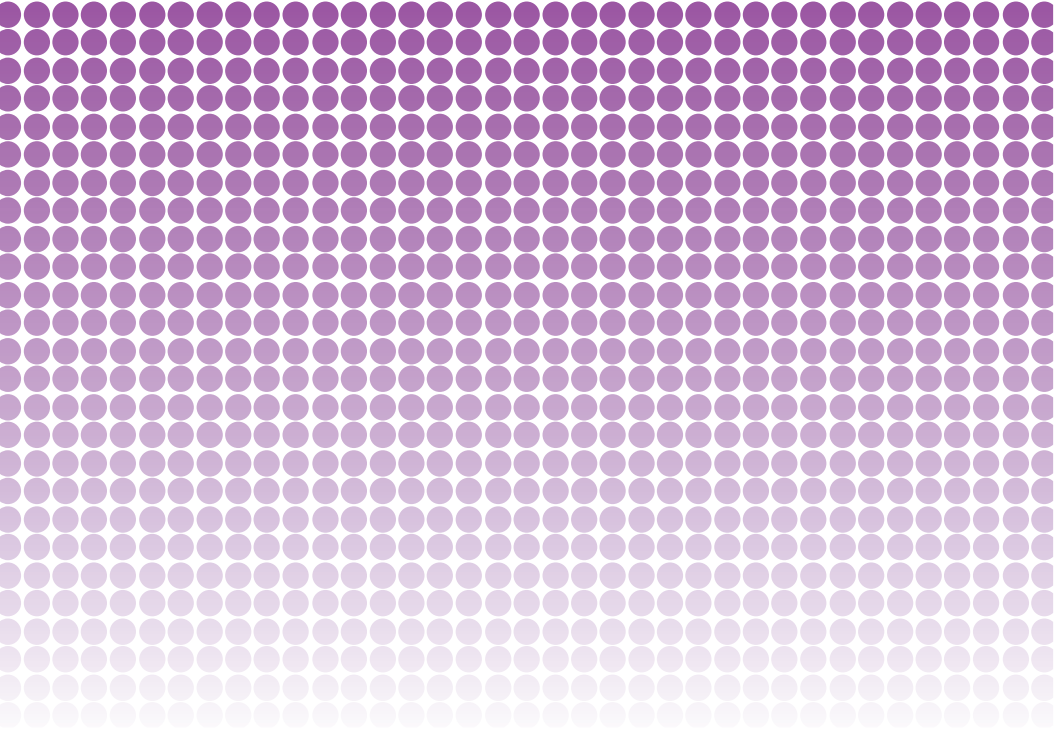
OPERATOR'S MANUAL

Peel Plate® EC

E. COLI AND COLIFORM

PP-ECHV

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



Contents

Kit Information	4
Introduction	4
Kit Contents, Storage, and Testing Conditions	4
Principle	5
Applicability.	5
Precautions	5

Sample Preparation	6
---------------------------	----------

Test Procedure	7
-----------------------	----------

Analysis of Results	8
----------------------------	----------

Interpretation of Results	8
----------------------------------	----------

Quality of Control	9
---------------------------	----------

Disposal	9
-----------------	----------

Technical Support	9
--------------------------	----------

Order Information	10
--------------------------	-----------

Warranty	11
-----------------	-----------

Kit Information

Introduction

Peel Plate® *E. coli* and coliform High Volume (EC HV) microbial tests detect and enumerate coliform bacteria, including *E. coli*, in 5 mL sample volumes. The method is applicable for determination of total coliform in dairy products (with the exception of cultured dairy) when incubated in the dark at $32 \pm 1^\circ\text{C}$ for 24 ± 2 hours. The method will colorimetrically differentiate *E. coli* from other coliform in non-dairy foods, environmental, and water samples incubated in the dark at $35 \pm 1^\circ\text{C}$ for 24 ± 2 hours. A 5 mL aliquot of a 1:5 sample dilution (or 5 mL on each of 2 plates of a 1:10 dilution) is added to the HV plate and is incubated at $32 \pm 1^\circ\text{C}$ for dairy samples and $35 \pm 1^\circ\text{C}$ for non-dairy samples. To test cultured dairy products, use the Peel Plate EC-CD-HV test. The Peel Plate EC HV test is intended for microbiological laboratories, but it 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 colony-forming units per 5 milliliter (CFU/5 mL) of test sample. The quantitative range for coliform, including *E. coli*, is defined as 1 to 154 CFU/5 mL.

Kit Contents, Storage, and Testing Conditions

A test kit (item code: PP-ECHV-25K) contains 25 tests in a foil bag containing a blue indicator desiccant. Store kits in foil bag refrigerated*, until expiration date.

Kits are not required to be shipped refrigerated.

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 tests will discolor yellow. Do not use discolored tests or tests from bags with pink/white desiccant indicator.

*Refrigerated is defined as 0 to 4.5°C and is required for US Certified labs.

Principle

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

Applicability

The Peel Plate EC HV test is applicable in liquid dairy (e.g. cream, flavored milks), solid dairy (e.g., ice cream), environmental surface sponges, and drinking and vegetable / fruit process water when a higher level of sensitivity of 1 to 154 CFU/5 mL is desired.

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 drafts.
- For best results, when testing semi-solid or solid samples containing curds or particulates, it is recommended to homogenize dilutions using stomacher bags with filters.
- Avoid hand contact with test samples and Peel Plate EC HV medium.

Sample Preparation

Dairy (Milk)	<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/5mL). <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.
Other Liquid and Solid Dairy	<ul style="list-style-type: none"> • Add 11 g of solid dairy (ice cream, heavy creams, etc.) to 99 mL of microbiologically suitable dilution blanks to reach countable range (1 to 154 CFU/5mL). • For milk powders and evaporated/condensed, reconstitute 11 g with 99 mL dilution blank and let any undissolved solids settle (no more than 3 minutes).
Environmental Swab	<ul style="list-style-type: none"> • Refer to Peel Plate Sample Preparation Addendum.
Bottled Water and Filtered Water Samples	<ul style="list-style-type: none"> • Water samples (5 mL) may be tested directly without preparation or after disinfectant neutralization with thiosulfate tablets. • For larger volume filtered water samples refer to Peel Plate Filtered Water Operators Manual.

Test Procedure



Step 1

- For ease of opening, use plates at room temperature.
- Label the test on the clear side.
- Invert and place test onto a level surface. Apply pressure with fingers to the back side of the cover and lift the tab.
- Pull the adhesive cover, exposing the culture disc. Leave cover adhered to back of plate.
- For best results, hold plates at room temperature prior to plating.



Step 2

- Rapidly dispense 5.0 mL of sample, or sample dilution, onto the center of the plate, keeping the pipet vertical. Expel pipet contents rapidly with even force and within 2 to 3 seconds.



Step 3

- Sample should diffuse towards the edges of plate. 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 the edges of the plate to ensure proper seal.
- Allow gel to set for 30 seconds before moving plate.



Step 4

- Incubate plates with adhesive cover down.
 - > Incubate in the dark at $32 \pm 1^\circ\text{C}$ for 24 ± 2 hours for milk and dairy products.
 - > Incubate in the dark at $35 \pm 1^\circ\text{C}$ for water, environmental swab, and non-dairy food samples.
- Plates can stack 12 high and will not affect plate heat transfer.

Analysis of Results

- At the end of incubation period, observe plates for colonies.
- Each colored spot, blue and red, represents one CFU. The sum of spots is reported as the total coliform CFU/5 mL of the diluted sample.
- Sum the results of one 5 mL plate of 1:5 diluted sample (or two 5 mL plates of 1:10 dilution) to calculate CFU/mL or CFU/gram of original sample.
- *E. coli* is a member of the coliform group and is differentiated colorimetrically by appearing dark blue, purple, or greenish color at 35°C . Other coliform bacteria will appear red (salmon) in color.
- 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/5 mL are considered in countable range, while counts outside that range are considered estimates. Samples with results outside of countable range (>154 CFU/mL) can be diluted and retested.

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*. Rather, it is classified as *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 $32 \pm 1^\circ\text{C}$ 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 HV 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 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 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 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 your local representative or Charm Sciences at +1.978.687.9200 or support@charm.com.

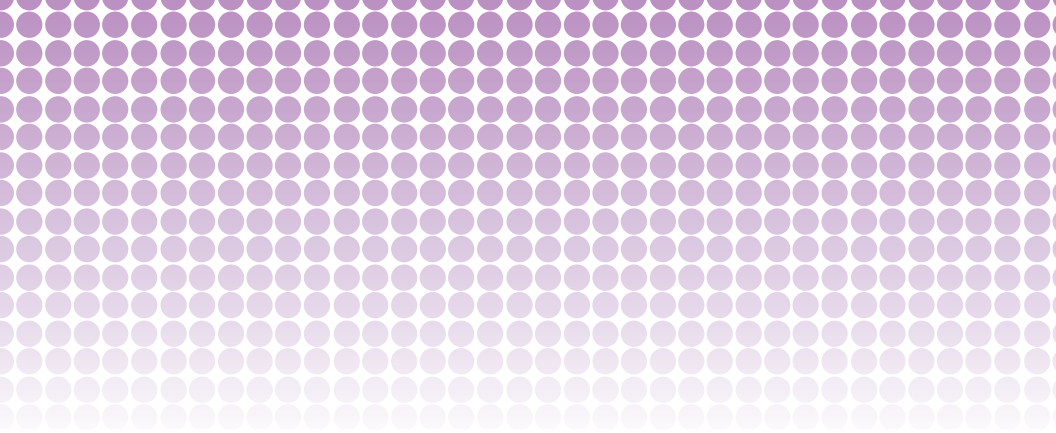
Order Information

Description	Quantity	Kit Code
Peel Plate EC HV	25	PP-ECHV-25K

High volume tests for aerobic count, *E. coli* / coliform, coliform count, 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.



OM-629-006



NOTHING WORKS LIKE A CHARM

659 Andover Street, Lawrence, MA 01843-1032, USA

T +1.978.687.9200 | **F** +1.978.687.9216 | **E** info@charm.com | **www.charm.com**

© 2025 Charm Sciences, Inc. Charm and Peel Plate are registered trademarks of Charm Sciences. See www.charm.com/patents for a list of U.S. issued patents and pending, published U.S. and PCT applications.

OM-629-006 Sep-2025