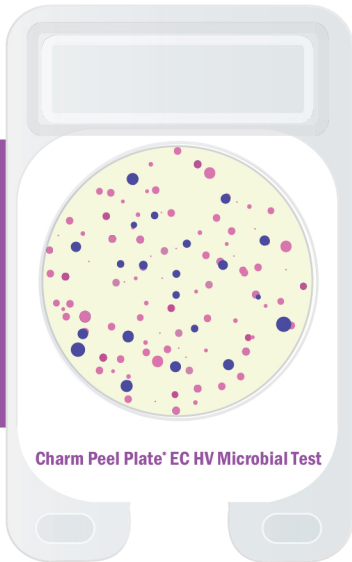




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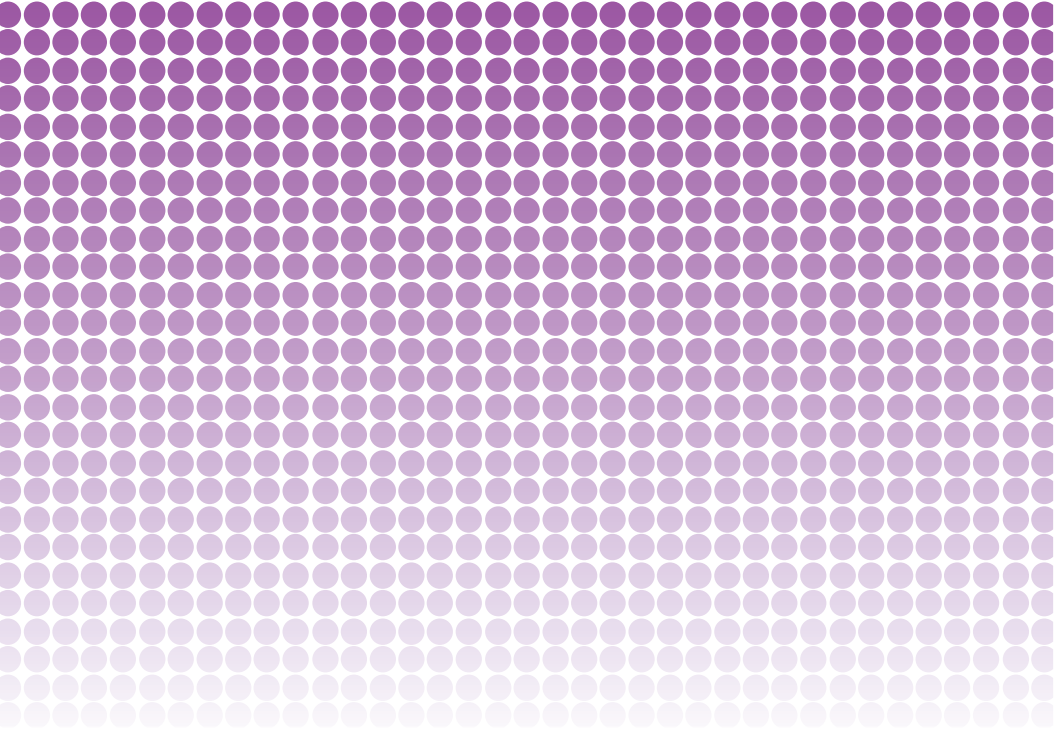


OPERATOR'S MANUAL



PeelPlate® EC HV
E. COLI AND COLIFORM HIGH VOLUME

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



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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 (except hard cheeses) 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, and $35 \pm 1^\circ\text{C}$ for non-dairy, samples. Fermented dairy products like yogurt require the addition of sodium bisulfite to the sample homogenate before testing, and hard cheeses require a different formulated Peel Plate test (EC-C-HV). The Peel Plate EC HV test is intended for microbiological laboratories, but 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 (Kit Code: PP-ECHV-25K) contains a foil bag with 25 Peel Plates EC HV formulation. Additionally, a bottle of Sodium Bisulfite Solution is supplied for preparing diluent for yogurt and other cultured dairy product testing besides hard cheeses. A 100 test kit (Kit Code: PP-ECHV-100K) contains 4 bags of 25 Peel Plate tests and 2 bottles of Sodium Bisulfite Solution. A separate formulated Peel Plate test is used for hard cheeses (Kit Code PP-EC-C-HV-100K).

Kits are not required to be shipped refrigerated.

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

Open the bag and perform testing in a clean dry testing area at ambient temperature. Remove the number of plates need for analysis. Reseal the bag using the zip closure to store unused tests. Moisture or heat or storage abused test will discolor yellow. Do not use discolored tests or tests from bags with pink/white desiccant indicator.

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. sour cream, ice cream, condensed whey), cultured dairy when homogenized in sodium bisulfite diluent (e.g. cottage cheese, yogurt), 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.
- 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, sour cream, creams, etc.) to 99 mL of microbiologically suitable dilution blanks heated to 40 to 45 °C to reach countable range (1 to 154 CFU/5mL). 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 Sodium Bisulfite Solution supplied with kit to the mix/homogenate and mix well. Alternatively dissolve 0.2 g sodium bisulfite in 99 mL dilution blank heated to 40 to 45 °C, add 11 g product, and mix/homogenize. For milk powders and evaporated/condensed, reconstitute 11 g with 99 mL dilution blank heated to 40 to 45 °C and let any undissolved solids settle (no more than 3 minutes). Cheeses (Parmesan, Provolone, Swiss, Romano, Cheddar, etc.) that produce a dark red background, require a separate Peel Plate method, EC-C-HV.
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

- 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.



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

- The sample should diffuse to the edges. Otherwise, immediately lift plate from surface and rotate to allow sample to spread to the edges of the plate.
- Re-apply the cover.
- Allow plate to sit 60 seconds before moving.



Step 4

- Incubate plates with adhesive cover down.
 - > Incubate in the dark at 32 ± 1 °C for 24 ± 2 hours for milk and dairy products. Yogurt requires an additional 24 hour incubation.
 - > Incubate in the dark at 35 ± 1 °C for water, environmental swab, and non-dairy food samples.
- Plates can stack 6 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.
- Cultured samples containing active LAB, e.g. yogurt, may present a reddish background and require an additional 24 hour incubation. Count distinct darker red and blue/purple colonies as coliform. Hard cheeses that produce a strong red background, like Parmesan, Romano, Swiss and Provolone, require a separate formulated Peel Plate EC-C-HV.

Interpretation of Results

- 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 °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**.

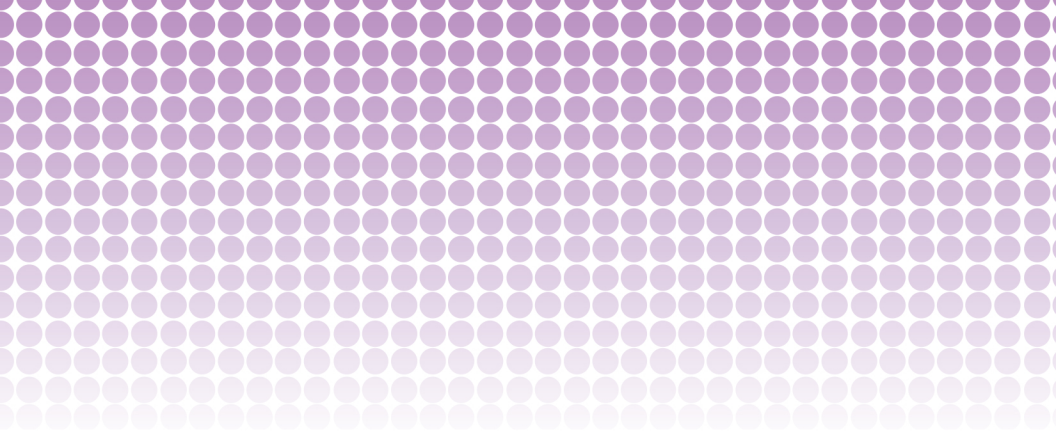
Order Information

Description	Quantity	Kit Code
Peel Plate EC HV	25	4150-11
	100	4150-30

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.



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