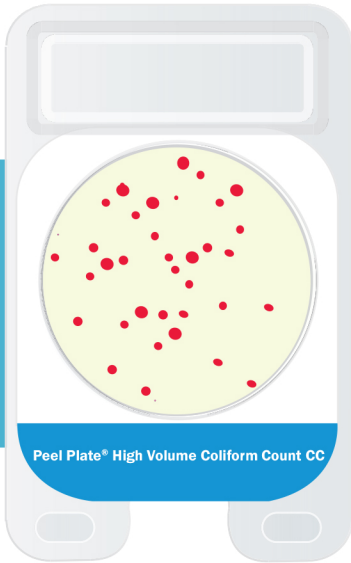




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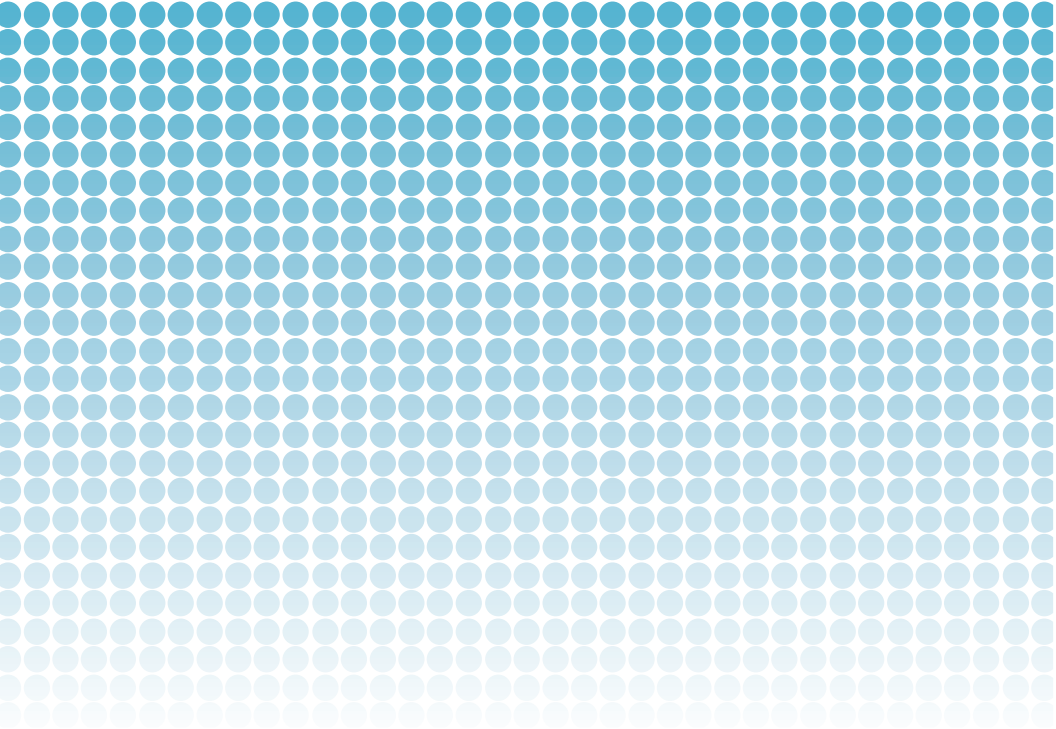


## OPERATOR'S MANUAL



**PeelPlate<sup>®</sup> CC HV**  
**COLIFORM HIGH VOLUME**

FOR DETECTION AND ENUMERATION OF TOTAL COLIFORM BACTERIA



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## Kit Information

### Introduction

Peel Plate® Coliform Count High Volume (CC HV) detects and enumerates coliform bacteria in 5 mL sample volumes. The method is applicable for detection of total coliform in dairy products, with the exception of hard cheeses, when incubated at  $32 \pm 1$  °C for  $24 \pm 2$  hours; or the detection of total coliform in non-dairy foods, environmental, and water samples incubated at  $35 \pm 1$  °C for  $24 \pm 2$  hours. To test hard cheeses use the Peel Plate CC-C-HV. A 5 mL aliquot of a 1:5 sample dilution (or 5 mL on each of 2 plates of a 1:10 sample dilution) is added to the HV plate and is incubated in the dark at  $32 \pm 1$  °C for dairy, and  $35 \pm 1$  °C for non-dairy samples. The Peel Plate CC 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 is defined as 1 to 154 CFU/5 mL.

### Kit Contents, Storage, and Testing Conditions

A 100 test kit (Kit Code: PP-CCHV-100K) contains 4 foil bags, each with 25 Peel Plate CC HV tests (formulation on 75 mm plates), and 2 30 mL bottles of Sodium Bisulfite Solution for preparing diluent for cultured dairy product testing. Hard cheeses use an alternatively formulated Peel Plate CC-C-HV kit.

Store test kit contents including plates in foil bags for up to 12 months in refrigerator or at room temperature for up to 1 month. Kits are not required to be shipped refrigerated.

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

## Principle

The Peel Plate CC HV medium is based on conventional coliform selective medium to support and colorimetrically differentiate coliform in test samples. The Peel Plate CC HV test contains the enzyme substrates salmon-gal (6-chloro-3-indolyl-B-D-galactopyranoside) used to detect  $\beta$ -galactosidase enzyme produced by coliform. The Peel Plate CC HV test also contains gelling and wicking agents which absorb and self-diffuse the sample.

## Applicability

The Peel Plate CC 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), and environmental surface sponges, when a higher level of sensitivity of 1 to 154 CFU/5 mL is desired. Hard cheeses require the use of an alternative formulated Peel Plate CC-C-HV.

## 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 draft-free.
- Avoid hand contact with test samples and Peel Plate CC HV medium.

## Sample Preparation

### Dairy (Liquid Cream)

- 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/5 mL).
  - To serially dilute, add 11 mL into 99 mL microbiologically suitable dilution blanks. Other automated dilution pipets and dilution schemes are acceptable, e.g. for 1:5 dilution add 25 mL to 99 mL dilution blank.

### 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/5mL). For 1:5 dilution add 25 g to 99 mL dilution blank.
- For fermented solid dairy (cottage cheese, yogurt, condensed whey, etc.) containing active lactic acid bacteria (LAB) culture.

- Add 11 g product to 99 mL dilution blank 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, add 11 g product, and mix/homogenize.
- For milk powders and evaporated/condensed, reconstitute 1:10 with diluent and let any undissolved solids settle (no more than 3 minutes).
- For hard cheeses, Parmesan, Cheddar, Swiss, Provolone, Muenster, etc, use alternatively formulated Peel Plate CC-C-HV.

## Environmental Swab

Refer to Peel Plate Sample Preparation Addendum.

### Peel Plate CC HV Procedure



- Step 1
- Apply pressure to back side of plate and pull up the cover tab.
  - Lift cover to expose the Peel Plate CC HV media.



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

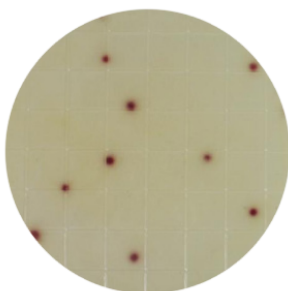


- Step 3
- Sample should diffuse to edges of plate. For viscous samples lift plate and rotate to ensure proper distribution of sample.
  - Re-apply the cover and avoid wrinkling.
  - Allow plate to sit 30 seconds before moving.



- Step 4
- Incubate plates in the dark with lid down as shown.
    - Incubate at  $32 \pm 1^\circ\text{C}$  for  $24 \pm 2$  hours for milk and dairy products. Yogurt and other cultured products require an additional 24 hour incubation.
    - Incubate at  $35 \pm 1^\circ\text{C}$  for 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 the incubation period, observe plates for colonies viewed through the bottom side of the plate.
- Each red spot, 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.
- 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 bisulfite addition with an additional 24 hour incubation. Cheeses that produce a strong red background need to use an alternative formulated Peel Plate CC-C-HV without bisulfite.

## Interpretation of Results

- The Peel Plate CC formulation is very similar to the Peel Plate EC formulation except the *E. coli* color indicator is removed. In the Peel Plate EC test inclusivity and exclusivity studies, 57 of 58 coliform inclusivity isolates were correctly detected, including all 17 *E. coli* strains. Of the 32 exclusivity strains evaluated, 31 were correctly excluded. The strain that was detected as coliform was, *Shigella sonnei*, ATCC 9290.
- The Peel Plate CC HV test has been evaluated in claimed foods, but has not been evaluated with all possible food products, food processes, testing protocols or with all possible microorganism strains.

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

## Order Information

The Peel Plate CC HV test is supplied in 25 (Kit code: PP-CCHV-25K) and 100 (Kit code: PP-CCHV-100K) test kits. These include sodium bisulfite concentrate for cultured dairy product preparation, but this concentrate may also be ordered separately (order code: BUF-NAS03). Kit codes for high volume Peel Plate for hard cheese testing are PP-CC-C-HV-25K and PP-CC-C-100K.

Peel Plate tests are also available in 1 mL sample volume tests in 100 and 1000 test kits for Aerobic Count, *E.coli*/coliform, Enterobacteriaceae, Yeast/Mold and Heterotrophic Plate Count. Refer to [www.charm.com](http://www.charm.com) for more information.

## 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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