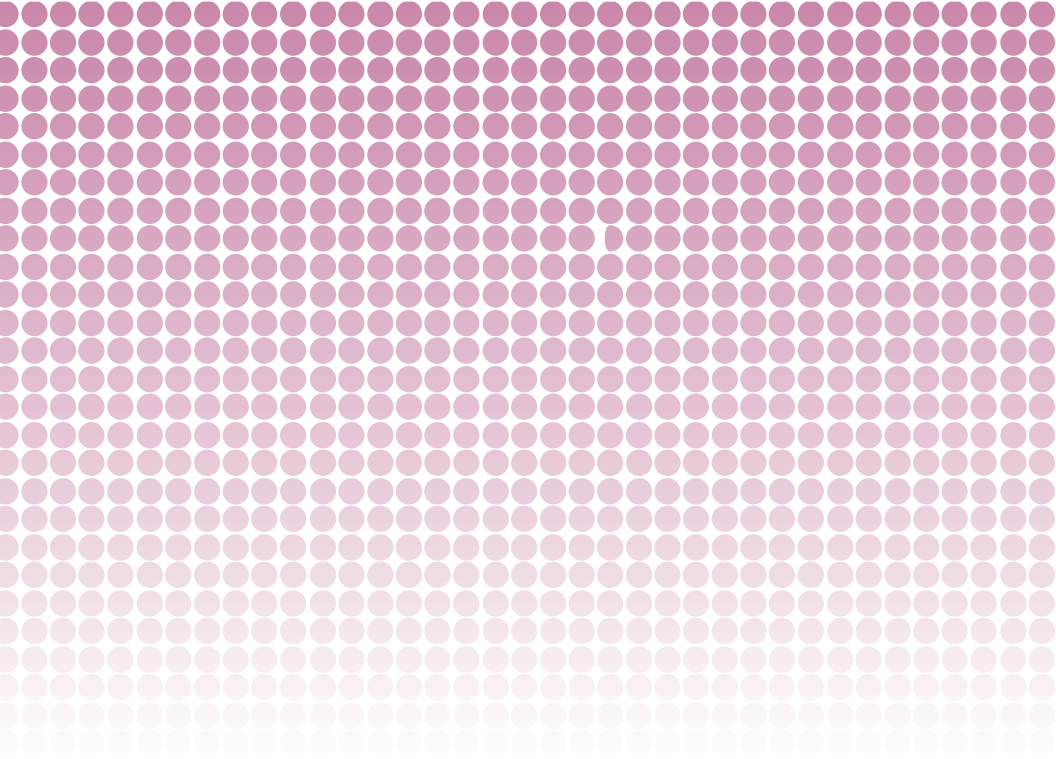


OPERATOR'S MANUAL



PP-AC

FOR DETECTION AND ENUMERATION OF
AEROBIC BACTERIA



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Kit Information

Introduction

Peel Plate AC microbial tests detect and enumerate aerobic bacteria in dairy, food, serial dilutions of food, and water samples. Sample or sample dilution is added in duplicate and incubated in the dark for 48 hours at $32^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for dairy products and $35^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for other matrices. Peel Plate AC 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 sensitivity is greater than 1 colony forming unit per milliliter (>1 CFU/mL) of test sample. The accurate quantitative range is defined as 25 to 250 CFU/plate and tested in duplicate.

Kit Contents, Storage, and Testing Conditions

A test kit (item code: PP-AC-50K) contains 50 tests in a foil bag containing a blue indicator desiccant. Store kits in foil bag refrigerated or at controlled room temperature (0 to 25°C), 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. Moisture or heat or storage abused test will discolor pink/red. Do not use discolored tests or tests from bags with pink/white desiccant indicator.

Principle

The Peel Plate AC test is based on Standard Methods agar to support and colorimetrically identify the growth of aerobic bacteria. Peel Plate AC media contains the enzyme substrate 2,3,5-triphenyltetrazolium chloride (TTC). TTC is reduced by living bacteria to produce red colored colonies. The media also contains gelling and wicking agents which absorb and diffuse the sample.

Applicability

The Peel Plate AC test has been certified by the AOAC Research Institute as Performance Tested Method 071501. This test kit's performance was reviewed by AOAC-RI and was found to perform to the manufacturer's specifications. The Peel Plate AC test has been validated at $32 \pm 1^\circ\text{C}$ for 48 ± 3 hours in raw cow milk laboratory pasteurized at $63 \pm 1^\circ\text{C}$ for 30 ± 1 minutes, raw goat milk, raw sheep milk, pasteurized whole milk 3.5% fat, pasteurized fat free skim milk, pasteurized chocolate milk 2% fat, pasteurized 20% cream, non-fat dried milk, UHT milk 1% fat, canned evaporated milk, canned sweetened condensed milk, vanilla ice cream, pasteurized strawberry milk, and pasteurized lactose reduced milk and found to not be significantly different from the standard plate count agar reference method. Aerobic count is not applicable to cultured dairy products.

The Peel Plate AC test has been validated at $35 \pm 1^\circ\text{C}$ for 48 ± 3 hours for non-dairy matrices including ground beef and turkey, liquid pasteurized eggs, dry ground dog food, environmental surface sponges, chicken carcass rinses and samples of 300 cm² sponged hog hides and was found to not be significantly different from the standard plate count agar reference method.

Samples should be 10-fold serially diluted into the countable range of 25 to 250 CFU/plate and tested in duplicate. from bags with pink/white desiccant indicator.

Precautions

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

- Perform tests with clean washed hands, and wear gloves if handling potential pathogenic bacteria.
- Test on a level surface in a clean area free of dust and draft-free.
- 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 medium.
- After plating, replace adhesive cover so it lays flat and free of wrinkles to avoid drying out the rehydrated medium during incubation.

Sample Preparation

Dairy	<ul style="list-style-type: none"> • Liquid dairy samples (raw milk and pasteurized dairy products) may be tested directly or serially diluted to a countable range (25 to 250 CFU/plate) and tested in duplicate. <ul style="list-style-type: none"> – To serially dilute, add 11 mL into 99 mL microbiologically suitable dilution blanks. Prepared Butterfield's buffer was used in validation. Other dilution options and dilution pipettors are acceptable.
Solid Dairy	<ul style="list-style-type: none"> • Add 11 g of solid dairy (ice cream, evaporated and sweetened condensed milk, etc.) to 99 mL of dilution blank to reach countable range (25 to 250 CFU/plate) and tested in duplicate. Do not test cultured products such as yogurt and cottage cheese. Mix well and allow separation of fat/foam. Test liquid portion as dairy within 3 minutes of mixing. • For milk powders and evaporated/condensed milks reconstitute to normal milk solids with dilution blank . Mix well and let solids settle. Test liquid portion as dairy within 3 minutes of mixing.
Ground Meat	<ul style="list-style-type: none"> • Add 50 g of ground meat to 450 mL of buffered peptone water (BPW), Butterfield's buffer, or microbiologically suitable dilution blank to extract sample. • Let particulates settle, and continue to dilute 10 mL of prior dilution in 90 mL of dilution blank to reach countable range (25 to 250 CFU/plate) and test in duplicate.
Liquid Egg	<ul style="list-style-type: none"> • Add 50 mL of liquid egg to 450 mL (100 mL to 900 mL is acceptable) of phosphate buffered saline or other microbiologically suitable dilution blank and continue to dilute 10 mL of prior dilution in 90 mL of dilution blank to reach countable range (25 to 250 CFU/plate).
Carcass Rinse and Sponge	<ul style="list-style-type: none"> • Refer to Peel Plate Sample Preparation Addendum.
Environmental Swab	<ul style="list-style-type: none"> • Refer to Peel Plate Sample Preparation Addendum.

Test Procedure



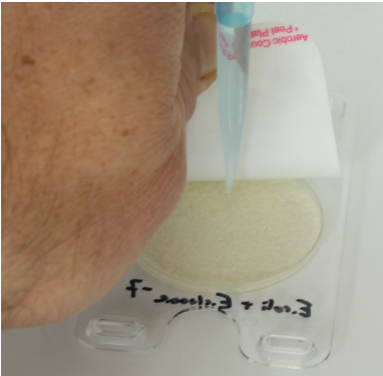
Step 1

- Label plate on clear side using marker. Do not mark or label the uplifted 47 mm² circular area.



Step 2

- Invert and place Peel Plate test onto a level surface. Apply pressure with fingers to the rear end of label or rectangular platform as shown.
- Lift tab and pull adhesive cover upwards and expose the dried media culture disc. Do not remove cover from back of plate.
- For best results, hold plates at room temperature prior to plating.



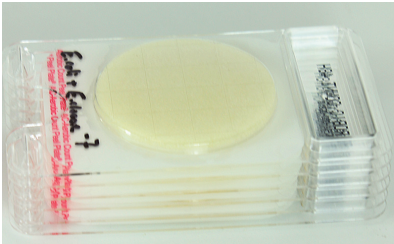
Step 3

- While holding cover up, and keeping plate flat on surface, **vertically dispense 1.0 mL onto the center of disc**. Expel pipet contents within 2 to 3 seconds 1 to 2 cm from surface.



Step 4

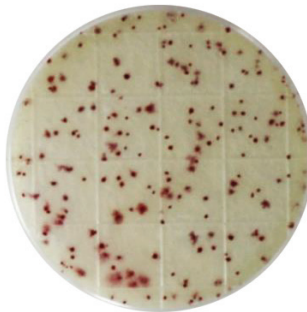
- Sample will diffuse to the edges of the disc. For viscous samples, gently tilt the plate towards the uplifted cover and rotate to ensure proper distribution of sample.
- Re-seal the adhesive cover without wrinkling. Press around edges of plate to ensure proper seal.
- Allow gel to set for 10 seconds before moving plate.
- By microbial testing convention, samples dilutions are analyzed in duplicate. Repeat Steps 1-4 with a second plate.



Step 5

- Incubate plates with clear side up, as shown. Incubate in the dark at $32 \pm 1^\circ\text{C}$ for 48 ± 3 hours for milk and dairy products, or at $35 \pm 1^\circ\text{C}$ for meat, eggs, foods, carcass, or environmental sponge samples.
- Plates can stack up to 20 high by aligning the feet and back rectangular platform. Stacking will not affect plate heat transfer.

Analysis of Results



- At the end of incubation period, observe plates for red/purple colonies as viewed through the clear side of plate. Each spot represents 1 CFU. The sum of red spots is reported as the CFU/mL or CFU/gram per dilution tested.
- In case of spreading bacteria, score a single CFU for each spot within the spread

growth. Blended colonies are scored as a single CFU.

- Average the duplicate test results to calculate CFU/plate. Multiply CFU/plate by the dilution to calculate a CFU/mL or CFU/g sample.
- Counts of 25 to 250 CFU/plate are considered quantitative results, while counts outside that range are considered estimates.
- Samples with results outside quantitative 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).

Optional Peel Plate Colony Counter:

- Insert completed test into the Peel Plate Colony Counter. Identify the plate as “Peel Plate AC”.
- Enter sample identity or verify identity has been populated.
- Press COUNT and CFU/mL will be displayed and recorded into memory with time/date. For more information, refer to Peel Plate Colony Counter instructions.

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 on Peel Plate test after incubation.
- **Negative Control:** Prepare Negative Control by autoclaving the appropriate dilution of test sample at 121°C for 15 minutes. Cool, then test 1.0 mL to verify no detectable bacteria in the Negative Control.
- **Positive Control:** Prepare Positive Control by spiking a sample with known titer bacterial culture. Dilute sample to countable range of 25 to 250 CFU/mL. Test 1.0 mL and verify detection after incubation to be within ± 50% of estimated titer bacterial culture.

Disposal

Collect microbiological cultures and reagents in biohazard bags and autoclave. 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.

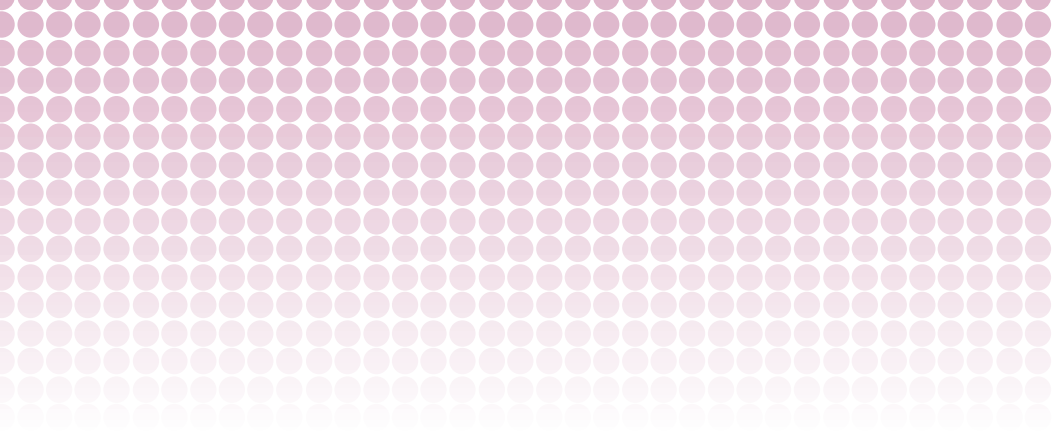
Order Information

Description	Quantity	Kit Code
Peel Plate AC	50	PP-AC-50K
	1000	PP-AC-1000K

Peel Plate tests for *E. coli* and coliform, total coliform, 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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