

Operator's Manual:



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 and incubated 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 units per milliliter (>1 CFU/mL) of test sample. The accurate quantitative range is defined as 25 to 250 CFU/mL.

Kit Contents, Storage, and Testing Conditions

A test kit (item code: PP-AC-100K) contains 100 tests, 50 in two desiccated foil bags containing a blue indicator desiccant. **Store kits in foil bag refrigerated*** for up to 12 months or at room temperature for up to 1 month.

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. **Plates held at room temperature for 1 hour or more will open more easily.** 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.

* Refrigerated is defined as 0 to 7°C or 0 to 4.5°C for US Certified Labs

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 $32^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 48 ± 3 hours in raw cow, goat, and sheep milk, and pasteurized whole milk, skim milk, chocolate milk, 20 % cream, non-fat dried milk, UHT milk, evaporated milk, sweetened condensed milk, vanilla ice cream, strawberry milk, and 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 $35^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 48 ± 3 hours for non-dairy matrices including ground beef and turkey, liquid eggs, dry dog food, environmental surface sponges, and carcass rinses 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/mL.

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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.
- 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/mL).• To serially dilute, add 11 mL into 99 mL microbiologically suitable dilution blanks. 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 microbiologically suitable dilution blank to reach countable range (25 to 250 CFU/mL). Do not test cultured products such as yogurt and cottage cheese.• For milk powders and evaporated/condensed milks reconstitute to normal milk solids with sterile water and let settle 3 minutes. Test as dairy.
Ground Meat	<ul style="list-style-type: none">• Add 50 g of ground meat to 450 mL of 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/mL).
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 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/mL).
Carcass Swab	<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.

Peel Plate AC Test Procedure



Step 1

- Label plate on clear side using marker or bar code strip. Do not mark or label the uplifted 47 mm 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. Leave cover adhered to back of plate.



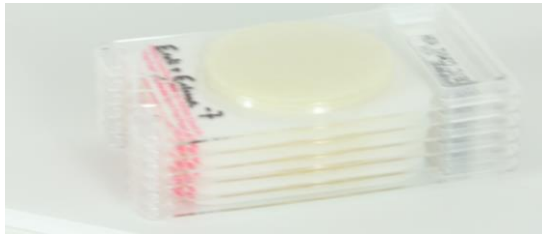
Step 3

- 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.
- Re-apply the adhesive cover without wrinkling. Press around edges of plate to ensure proper seal.



- Step 5**
- Incubate plates with clear side up, as shown. Incubate at **32 ± 1 °C for 48 ± 3 hours** for milk and dairy products, **or at 35 ± 1 °C** for meat, eggs, foods, carcass, or environmental sponge samples.
 - Plates can stack up to 20 high by aligning the 2 feet and 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.
- Multiply CFU/mL by the dilution to calculate a CFU/mL or CFU/g sample.
- Counts of 25 to 250 CFU/mL are considered quantitative results, while counts outside that range are considered estimates.
- Samples with results outside quantitative range should be diluted and retested.
- An estimated count of plates with greater than 250 colonies or Too Numerous to Count (TNTC) may be done using the etched grids. Pick a 1 cm grid with representative growth and count, or pick 5 grids and take average, and multiply by 17.4, the area of the plate. This is the estimate of the counts per plate. This would then be multiplied by the dilution factor for eCFU/mL or g sample.

Optional Peel Plate Reader:

- Insert completed test into the Peel Plate Reader.
- Enter sample identity or verify bar code identity has been populated.
- Start by identifying the plate as Peel Plate AC microbial test.
- CFU/mL will be displayed and recorded into memory with time/date. For more information, refer to Peel Plate Reader 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 $\pm 50\%$ 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	100	PP-AC-100K
	1000	PP-AC-1000K

Peel Plate tests for *E. coli* and coliforms, yeast and mold, and heterotrophic bacteria are also available. Visit Charm Sciences' website at www.charm.com to learn more.

Warranty

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