OPERATOR’S MANUAL

Peel Plate® SA
STAPHYLOCOCCUS AUREUS

FOR DETECTION AND ENUMERATION OF STAPHYLOCOCCUS AUREUS BACTERIA IN FOOD, SERIAL DILUTIONS OF FOOD, AND ENVIRONMENTAL SPONGE SAMPLES
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Kit Information

Introduction

Peel Plate SA (*Staphylococcus aureus*) tests detect and enumerate Staphylococcal bacteria in food, serial dilutions of food, and environmental sponge samples (refer to Applicability for validated matrices). Sample or sample dilution is added and incubated for 24 to 48 hours at 35-37 °C.

Peel Plate SA tests are intended for microbiological laboratories, but may also be used by food quality stakeholders such as farmers, milk processors, and ready-to-eat food producers. The method sensitivity is greater than 1 colony forming units per milliliter (>1 CFU/mL) of test sample.

Kit Contents, Storage, and Testing Conditions

A test kit (item code: PP-SA-100K) contains 100 tests - 50 tests in two desiccated foil bags containing a blue indicator desiccant.

Kits are not required to be shipped refrigerated.

*Store kits in foil bag refrigerated* until expiration date.

Open bag and perform testing in a clean dry testing area at ambient temperature. Remove number of plates need for analysis. **Tests held at ambient temperature for 1 hour or more will open more easily.** Reseal the bag using the zip closure to store unused tests. Moisture, heat, or storage abused tests will discolor yellow. Do not use discolored tests or tests from bags with a pink/white desiccant indicator.

*Refrigeration is defined as 0 to 4.5 °C*

Principle

The Peel Plate SA test is based on Baird Parker selective agar, and multiple colorimetric enzyme substrates to support growth and colorimetrically identify the growth of *Staphylococcus aureus* bacteria. The media also contains gelling and wicking agents which absorb and diffuse the sample. The method will provide a presumptive positive *Staphylococcus aureus* result that can then be confirmed following traditional confirmation procedures as outline in the FDA-BAM (Bacteriologic Analytical Manual).
Applicability

The Peel Plate SA test is applicable to multiple food matrices incubated in the dark at 35-37 °C for 24 to 48 hours.

Samples should be 10-fold serially diluted into the FDA-BAM defined countable range of 20 to 200 CFU/mL.

Precautions

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

- Raw foods, processed foods, animal products, and their contact surfaces may contain harmful microorganisms or pathogens such as *Staphylococcus aureus*, *Listeria monocytogenes*, hemorrhagic *E. coli*, and *Salmonella enteritidis*.

- Take care in handling raw food and animal products, and the developed tests as they may contain these potential hazardous microorganisms.

- If direct contact or spillage occurs, thoroughly wash affected area using detergent and water for skin and clothing; and disinfectant for other surfaces. If eye or mouth contact rinse thoroughly. If there is a subsequent illness, irritation or infection contact a physician.

- Avoid contact with tests samples and Peel Plate SA medium. Perform tests and handle developed tests wearing personal protective equipment such as eye wear, lab coats and gloves.

- Perform test on a level surface in a clean area, free of dust and blowing air.

- After plating, re-seal adhesive cover so that it lays flat with no wrinkles to avoid drying out the rehydrated medium during incubation.

- To reduce the potential of sample cross contamination, wash and disinfect, glassware and work area in contact with foods and developed tests.
## Sample Preparation

| Liquid Food | • Liquid food samples (milk, pasteurized liquid dairy products, eggs) may be tested directly or serially diluted to less than 200 CFU/mL.  
|            | ▶ To serially dilute, add 25 mL sample into 225 mL microbiologically suitable dilution blanks. Other automated dilution pipets and 1 part sample to 9 part buffer dilution schemes are acceptable.  
|            | • For milk powders and evaporated/condensed milks reconstitute to normal milk solids with sterile water and let settle 3 minutes. Test as liquid food. |
| Solid Food | • Add 25 g of solid food (processed meats, sandwiches, salads, raw fish, dairy products, fresh fruit and vegetables, etc) to 225 mL of microbiologically suitable dilution blank and serially dilute as necessary to less than 200 CFU/mL.  
|            | • Homogenize or stomach for 2 minutes. Let particulates settle, and continue to dilute 10 mL of prior dilution in 90 mL (or 11 to 99 mL) of dilution blank to reach less than 200 CFU/mL. Other 1 part to 9 part dilution schemes are acceptable. |
| Environmental Swab | • Refer to Peel Plate Sample Preparation Addendum. |
Peel Plate SA Test Procedure

Step 1

• For ease of opening, use plates at room temperature.

• 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 test onto a level surface. Apply pressure with fingers to the back platform as shown and lift tab.

• Pull the adhesive cover exposing the 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 in 2 to 3 seconds while 1 to 2 cm from surface.

Step 4

• Sample will diffuse to the edges of the disc.

• Re-seal the adhesive cover without wrinkling. Press around edges of plate to ensure proper seal.
Step 5

- Incubate plates in the dark with clear side up, as shown. Incubate at **35-37 °C for 24 to 48 hours**.
- 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 24 Hour Incubation Check**

- Check plates for growth at 24 hours. If no growth, test is complete. If growth is present, continue incubating for additional 24 hours (48 hours total). Any purple or green(bluish) colonies with a white center after 24 hour incubation are presumptive positive for *Staphylococcus aureus*. Mark all presumptive colonies at 24 hours and continue incubation.

**At 48 Hour Incubation Check**

- At 48 hours, all purple colonies with or without a white center are presumptive positive for *Staphylococcus aureus*. Confirm that any green colonies with a white center at 24 hours are now purple. Count the total number of purple colonies on the plate and report as total presumptive positive for *Staphylococcus aureus*. Any red or green(bluish) colonies at 48 hours are negative and not counted as *Staphylococcus aureus*. If a colony is not purple (with or without a white center) at 48 hours it is not *Staphylococcus aureus*. 
• 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 reciprocal to calculate a CFU/mL or CFU/g sample.

• Counts of 20 to 200 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.

• An estimated count of plates with greater than 200 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 CFU/mL or g sample.

Optional Confirmation of Presumptive Positive Colonies
Confirmation of presumptive positives may follow the traditional BAM method. Use a plate with count between 20 and 200 CFU/plate. Plates less than 20 may be used, but plates greater than 200 may underestimate and sample should be additionally diluted and tested. Count each type of morphology on the plate for example purple, green and red and with and without white spots. Pick two or more of each type of bacterial colony. Test for a coagulase positive activity (method not supplied with Peel Plate). Only morphology presenting a strong coagulase positive reaction are confirmed as *Staphylococcus aureus*. Refer to FDA-BAM [https://www.fda.gov/Food/Food-ScienceResearch/LaboratoryMethods/ucm071429.htm](https://www.fda.gov/Food/Food-ScienceResearch/LaboratoryMethods/ucm071429.htm) for additional testing that may be done to confirm presumptive positive samples. The count of morphology that confirm positive are summed for the confirmed *Staphylococcus aureus* CFU/mL-g.

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 of *Staphylococcus aureus* bacterial culture. Dilute sample to countable range of 20 to 200 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**

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<tr>
<th>Description</th>
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<td>Peel Plate SA</td>
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Peel Plate tests for *E. coli* and coliforms, coliform count, aerobic bacteria, 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, NONINFRINGEMENT, 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.