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
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 ºC

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 outlined in the FDA-BAM (Bacteriological Analytical Manual).
Applicability
The Peel Plate SA test is applicable to multiple food matrices. Tests are incubated in the dark at 35-37 °C for 24 to 48 hours.

Samples should be 10-fold serially diluted into the countable range of 1 to 150 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) may be tested directly or serially diluted to less than 150 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 150 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 150 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 \(35-37\, ^\circ\text{C}\) 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 the end of the 24 hour period, observe plates for colonies as viewed through the clear side of plate. If there is no growth, the test is complete. Each purple spot or green (bluish) spot with a white center represents a presumptive positive of 1 CFU of \textit{Staphylococcus aureus}. Green (bluish) and or red spots without white centers are not \textit{Staphylococcus aureus} but should be incubated an additional 24 hours.

- At 48 hours, all purple colonies, larger than a small pin point, with or without a white center are presumptive positive for \textit{Staphylococcus aureus}. Do not count any purple colonies that are only a small pin point, as these may be non-aureus staphyloccoccus. Count the total number of purple colonies, larger than a small pin point, on the plate and report as total presumptive positive for \textit{Staphylococcus aureus}. Confirm that any green colonies with a white center at 24 hours have changed to purple.

Positive \textit{Staphylococcus aureus} and questionable colonies may be confirmed with catalase and coagulase positive results.

Any red or green (bluish) colonies at 48 hours are negative and not counted as \textit{Staphylococcus aureus}. 
With some pre-enriched samples, plates may overgrow producing more than 150 CFU per plate, producing smaller colonies. Overgrown plates with purple colonies should be further diluted and plated into the 0-150 CFU range or treated as questionable and confirmed by catalase and coagulase tests.

- 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 1 to 150 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 150 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 1 and 150 CFU/plate. Plates less than 10 CFU may be used, but plates greater than 150 may underestimate actual count and sample should be additionally diluted and tested. Count each typical morphology on the plate for example purple with and without white spots. Pick one or more of each type of colony. Test for a catalase and 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 guidelines 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 or 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, preferably of the matrix being routinely tested, with known titer of *Staphylococcus aureus* bacterial culture. Dilute sample to countable range of 1 to 150 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

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