






















Petrifilm™

6446/6490/6491/
6492/6493

Product Instructions

-  (EN) Staph Express System
-  (FR) Système Staph Express
-  (DE) Staph Express System
-  (IT) Sistema Staph Express
-  (ES) Sistema Staph Express
-  (NL) Staph Express Systeem
-  (SV) Staph Express System
-  (DA) Staph Express system
-  (NO) Staph Express-system
-  (FI) Staph Express järjestelmä
-  (PT) Sistema Staph Express
-  (EL) Σύστημα Staph Express
-  (PL) System do oznaczania liczby *Staphylococcus*
-  (RU) Система для экспресс-подсчета стафилококков
-  (TR) Staph Express Sistemi
-  (JA) 黄色ブドウ球菌エクスプレス測定用システム
-  (ZH) 金黄色葡萄球菌确认系统
-  (TH) Staph Express System
-  (KO) 황색포도상구균용 시스템

STX
Staph Express

Product Instructions

Staph Express System

Product Description and Intended Use

The 3M™ Petrifilm™ Staph Express (STX) System consists of a 3M™ Petrifilm™ Staph Express Count (STX) Plate and a 3M™ Petrifilm™ Staph Express (STX) Disk, which are packaged separately. The 3M Petrifilm STX Plate is a sample-ready-culture medium system which contains a cold-water-soluble gelling agent. The chromogenic, modified Baird-Parker medium in the plate is selective and differential for *Staphylococcus aureus* but may also indicate *Staphylococcus hyicus* (*S. hyicus*) or *Staphylococcus intermedius* (*S. intermedius*). The 3M Petrifilm STX Disk contains toluidine blue-O that facilitates the visualization of deoxyribonuclease (DNase) reactions. DNase-positive organisms detected on the 3M Petrifilm STX Plate include *Staphylococcus aureus* (*S. aureus*). The 3M Petrifilm STX Plates and 3M Petrifilm STX Disks are used for the enumeration of DNase positive *Staphylococcus* species in the food and beverage industries. 3M Petrifilm STX Plate and 3M Petrifilm STX Disk components are decontaminated though not sterilized.

3M Food Safety is certified to International Organization for Standardization (ISO) 9001 for design and manufacturing. 3M Petrifilm STX System has not been evaluated with all possible food products, food processes, testing protocols or with all possible microorganism strains.

Safety

The user should read, understand, and follow all safety information in the instructions for the 3M Petrifilm STX Plate and 3M Petrifilm STX Disk. Retain the safety instructions for future reference.

⚠ **WARNING:** Indicates a hazardous situation, which, if not avoided, could result in death or serious injury and/or property damage.

⚠ WARNING

To reduce the risks associated with exposure to biohazards and environmental contamination:

- Follow current industry standards and local regulations for disposal of biohazardous waste.

To reduce the risks associated with release of contaminated product:

- Follow all product storage instruction contained in the instructions for use.
- Do not use beyond the expiration date.

To reduce the risks associated with bacterial infection and workplace contamination:

- Perform 3M Petrifilm STX Plate and 3M Petrifilm STX Disk testing in a properly equipped laboratory under the control of a skilled microbiologist.
- The user must train personnel in current proper testing techniques: for example, Good Laboratory Practices¹, ISO 17025³ or ISO 7218².

To reduce the risks associated with misinterpretation of results:

- 3M has not documented 3M Petrifilm STX Plates and 3M Petrifilm STX Disk for use in industries other than food and beverage. For example, 3M has not documented 3M Petrifilm STX Plates and 3M Petrifilm STX Disk for testing water, pharmaceuticals, or cosmetics.
- Do not use 3M Petrifilm STX Plates and 3M Petrifilm STX Disk in the diagnosis of conditions in humans or animals.
- Do not use 3M Petrifilm STX Plates and 3M Petrifilm STX Disk for U.S.-recognized laboratory pasteurized counts.
- Acceptance of the 3M Petrifilm STX Plate and 3M Petrifilm STX Disk method for the testing of water per an accepted local government regulation is at the sole discretion and responsibility of the end-user.
- 3M Petrifilm STX Plate and 3M Petrifilm STX Disk do not differentiate any one microorganism strain from another.

Consult the Safety Data Sheet for additional information.

If you have questions about specific applications or procedures, please visit our website at www.3M.com/foodsafety or contact your local 3M representative or distributor.

User Responsibility

Users are responsible for familiarizing themselves with product instructions and information. Visit our website at www.3M.com/foodsafety, or contact your local 3M representative or distributor for more information.

When selecting a test method, it is important to recognize that external factors such as sampling methods, testing protocols, sample preparation, handling, and laboratory technique may influence results.

It is the user's responsibility in selecting any test method or product to evaluate a sufficient number of samples with the appropriate matrices and microbial challenges to satisfy the user that the chosen test method meets the user's criteria.

It is also the user's responsibility to determine that any test methods and results meet its customers' and suppliers' requirements.

As with any test method, results obtained from use of any 3M Food Safety product do not constitute a guarantee of the quality of the matrices or processes tested.

Limitation of Warranties / Limited Remedy

EXCEPT AS EXPRESSLY STATED IN A LIMITED WARRANTY SECTION OF INDIVIDUAL PRODUCT PACKAGING, 3M DISCLAIMS ALL EXPRESS AND IMPLIED WARRANTIES, INCLUDING BUT NOT LIMITED TO, ANY WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR USE. If any 3M Food Safety Product is defective, 3M or its authorized distributor will, at its option, replace or refund the purchase price of the product. These are your exclusive remedies. You must promptly notify 3M within sixty days of discovery of any suspected defects in a product and return it to 3M. Please call Customer Service (1-800-328-1671 in the U.S.) or your official 3M Food Safety representative for a Returned Goods Authorization.

Limitation of 3M Liability

3M WILL NOT BE LIABLE FOR ANY LOSS OR DAMAGES, WHETHER DIRECT, INDIRECT, SPECIAL, INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING BUT NOT LIMITED TO LOST PROFITS. In no event shall 3M's liability under any legal theory exceed the purchase price of the product alleged to be defective.

Storage

Plate Storage

Store unopened 3M Petrifilm STX Plate pouches refrigerated or frozen at temperatures lower than or equal to 8°C (46°F). Just prior to use, allow unopened pouches to come to room temperature before opening. Return unused 3M Petrifilm STX Plates to pouch. Seal by folding the end of the pouch over and applying adhesive tape. **To prevent exposure to moisture, do not refrigerate opened pouches.** Store resealed pouches in a cool dry place for no longer than four weeks. It is recommended that resealed pouches of 3M Petrifilm STX Plates be stored in a freezer (see below) if the laboratory temperature exceeds 25°C (77°F) and/or the laboratory is located in a region where the relative humidity exceeds 50% (with the exception of air-conditioned premises).

Disk Storage

3M Petrifilm STX Disks are individually packaged within a foil pouch. They are sensitive to both moisture and light. Upon receipt, store unopened pouches of 3M Petrifilm STX Disks refrigerated or frozen at temperatures lower than or equal to 8°C (46°F). Remove only those individually packaged 3M Petrifilm STX Disks that will be used immediately; store the remaining 3M Petrifilm STX Disks in the foil pouch by folding the end of the pouch and applying adhesive tape. **To prevent exposure to moisture, do not refrigerate opened pouches.** Store resealed pouches in a cool, dry place for no longer than six months. It is recommended that resealed pouches of 3M Petrifilm STX Disks be stored in a freezer (see below) if the laboratory temperature exceeds 25°C (77°F) and/or the laboratory is located in a region where the relative humidity exceeds 50% (with the exception of air-conditioned premises).

Freezer Storage

To store opened pouches of 3M Petrifilm STX Plates and 3M Petrifilm STX Disks in a freezer, place 3M Petrifilm STX Plates and 3M Petrifilm STX Disks in a sealable container. To remove frozen 3M Petrifilm STX Plates and 3M Petrifilm STX Disks for use, open the container, remove the 3M Petrifilm STX Plates and 3M Petrifilm STX Disks that are needed and immediately return remaining 3M Petrifilm STX Plates and 3M Petrifilm STX Disks to the freezer in the sealed container. 3M Petrifilm STX Plates and 3M Petrifilm STX Disks should not be used past their expiration date. Do not store open pouches in a freezer with an automatic defrost cycle, as this could damage the 3M Petrifilm STX Plates due to repeated exposure to moisture.

Do not use 3M Petrifilm STX Plates and 3M Petrifilm STX Disks that show discoloration. Expiration date and lot number are noted on each package of 3M Petrifilm STX Plates and 3M Petrifilm STX Disks. The lot number is also noted on individual 3M Petrifilm STX Plates and on the individual 3M Petrifilm STX Disk packages.

△ Disposal

After use, 3M Petrifilm STX Plates may contain microorganisms that may be a potential biohazard. Follow current local, regional, national and industry standards for disposal.

Instructions for Use

Follow all instructions carefully. Failure to do so may lead to inaccurate results.

Sample Preparation

1. Use appropriate sterile diluents:

Butterfield's phosphate buffered dilution water⁴, peptone salt diluent⁵, 0.1% peptone water, buffered peptone water⁵, quarter-strength Ringer's solution, saline solution (0.85-0.90%), bisulfite-free letheen broth, or distilled water. See section "**Specific Instruction for Validated Methods**" for specific requirements.

Do not use diluents containing citrate, bisulfite, or thiosulfate with 3M Petrifilm STX Plates; they can inhibit growth. If citrate buffer is indicated in the standard procedure, substitute warmed to 40-45°C (104-113°F) Butterfield's phosphate buffered dilution water or peptone salt diluent.

Do not use dipotassium hydrogen phosphate as the DNase reaction may be inhibited.

Select commercially made buffered peptone water media formulated to meet the requirements of ISO 6887 (buffered peptone water (BPW) (ISO)) may inhibit the DNase reaction resulting in no pink zone formation when the 3M Petrifilm STX Plate is used with the 3M Petrifilm STX Disk. It is important to verify the performance of the 3M Petrifilm STX Disk with the diluent chosen for sample preparation. Failure to do so, may result in false negatives.

2. Blend or homogenize sample.
3. For optimal growth and recovery of microorganisms, adjust the pH of the sample suspension to 6 - 8. For acidic products, adjust the pH with 1N NaOH. For alkaline products, adjust the pH with 1N HCl.

Plating

1. Place the 3M Petrifilm STX Plate on a flat, level surface.
2. Lift the top film and with the pipette perpendicular dispense 1 mL of sample suspension onto the center of bottom film.
3. Roll the top film down onto the sample to prevent trapping air bubbles.
4. Place the 3M™Petrifilm™ Flat Spreader (Catalog #6425) with the flat side down on the center of the plate. Press gently on the center of the spreader to distribute the sample evenly. Spread the inoculum over the entire 3M Petrifilm STX Plate growth area before the gel is formed. Do not slide the spreader across the film.
5. Remove the spreader and leave the 3M Petrifilm STX Plate undisturbed for at least one minute to permit the gel to form.

Incubation

Incubate 3M Petrifilm STX Plates in a horizontal position with the clear side up in stacks of no more than 20 3M Petrifilm STX Plates.

Incubate 3M Petrifilm STX Plates for 24 ± 2 hours at 35°C ± 1°C or 37°C ± 1°C (temperature based on validated references). See section "**Specific Instruction for Validated Methods**" for specific requirements.

Interpretation

1. Count 3M Petrifilm STX Plates with a standard colony counter or other illuminated magnifier. Do not count colonies on the foam dam since they are removed from the selective influence of the medium.
2. Observe colony colors.
 - a. If no colonies or only red-violet colonies are present after 24 ± 2 hours, count red-violet colonies as *S. aureus*, *S. hyicus*, or *S. intermedius*; the test is complete. Use of the 3M Petrifilm STX Disk is not necessary.
 - b. If a confirmation test is required or if any colony colors besides red-violet – for example, black or blue-green – are present, use a 3M Petrifilm STX Disk (see steps 3-11). Black colonies may be stressed microorganisms.

NOTE: High levels of phosphatase, an enzyme that occurs naturally in some foods, such as raw milk products, may cause the entire 3M Petrifilm STX Plate to turn pink and can obscure the red-violet colonies. If this occurs, dilute your sample further.

3. Remove an individually-packaged 3M Petrifilm STX Disk from its pouch and allow it to come to room temperature. Then remove the 3M Petrifilm STX Disk from its individual package by peeling the package to expose the 3M Petrifilm STX Disk's tab, grasping the tab, and pulling the 3M Petrifilm STX Disk out.

4. Lift the top film of the 3M Petrifilm STX Plate and place the 3M Petrifilm STX Disk in the well of the 3M Petrifilm STX Plate so that the tab remains outside the well.

NOTE: Occasionally the gel may split when the top film is lifted. Performance of the 3M Petrifilm STX Plate is not affected by gel splitting because the 3M Petrifilm STX Disk is coated on both sides.

5. Lower the top film.
6. Apply pressure by sliding a finger firmly across the entire 3M Petrifilm STX Disk area (including edges) to ensure uniform contact of the 3M Petrifilm STX Disk with the gel and to eliminate any air bubbles.

NOTE: If too much pressure is applied, the gel may be disturbed, and zones may not be distinct. NOTE: If too little pressure is applied, the gel may dry, and zones may not form.

7. Place the disked 3M Petrifilm STX Plates in stacks of no more than 20 3M Petrifilm STX Plates and incubate at $35^{\circ}\text{C} \pm 1^{\circ}\text{C}$ or at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for at least 60 minutes and no longer than 3 hours. Check the 3M Petrifilm STX Plates periodically—the DNase reaction may give final results in less than 3 hours. If all of the colonies have pink zones, the testing is complete and the colonies can be counted. If some colonies do not have zones then the 3M Petrifilm STX Plate must be incubated for the full 3 hours.

NOTE: If the 3M Petrifilm STX Plates cannot be counted within 1 hour of removal from the incubator they may be stored for later enumeration by freezing in a sealable container at temperatures less than or equal to minus 15°C (5°F) for no longer than one week. Allow 3M Petrifilm STX Plates to reach room temperature, and then use the 3M Petrifilm STX Disk as described above.

NOTE: Never freeze disked 3M Petrifilm STX Plates for later interpretation.

8. Count all pink zones whether or not colonies are visible. Pink zones are usually associated with *S. aureus* but may indicate *S. hyicus* or *S. intermedius*. Colonies not associated with a pink zone are not DNase producing Staphylococci, and should not be counted. If the entire disked area is pink with no distinct zones, large numbers of DNase producing colonies are present. Record the result as too numerous to count (TNTC) and dilute the sample further to obtain a more accurate count.
9. When necessary, colonies may be isolated for further identification. Lift the top film and pick the colony from the gel. If a 3M Petrifilm STX Disk is covering the gel, peel the 3M Petrifilm STX Disk away using a forceps and then pick the colony from the gel. Dispose of the 3M Petrifilm STX Disk by following current industry standards and sterilize the forceps.

For further information refer to the appropriate 3M Petrifilm Staph Express Count Plate and Disk “Interpretation Guide.” If you have questions about specific applications or procedures, please visit our website at www.3M.com/foodsafety or contact your local 3M representative or distributor.

Specific Instructions for Validated Methods

AOAC® Official MethodsSM (2003.07 3M Petrifilm STX Plate Method for the Enumeration of *Staphylococcus aureus* in Selected Types of Processed and Prepared Foods)

AOAC® Official MethodsSM (2003.08 3M Petrifilm STX Plate Method for the Enumeration of *Staphylococcus aureus* in Selected Dairy Foods)

AOAC® Official MethodsSM (2003.11 3M Petrifilm STX Plate Method for the Enumeration of *Staphylococcus aureus* in Selected Meat, Seafood, and Poultry)

Incubate 24 hours \pm 2 hours at $35^{\circ}\text{C} \pm 1^{\circ}\text{C}$ or $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$

- If no colonies or only red-violet colonies appear, test is complete, no need to use disk. Count red- violet colonies as *S. aureus*.
- If colony colors besides red-violet appear, insert disk and re-incubate 1 to 3 hours at $35^{\circ}\text{C} \pm 1^{\circ}\text{C}$ or $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$. Count pink zones as *S. aureus*.

NF Validation by AFNOR Certification

NF Validation certified method in compliance with ISO 16140⁶ in comparison to ISO 6888-1⁷ (3M -01/9-04/03A) and ISO 6888-2⁸ (3M-01/9-04/03B)

Scope of the validations: All human foods and pet food

Use the following details when implementing the above Instructions for Use:

Sample preparation

Use only ISO listed diluents.

Incubation

Incubate 3M Petrifilm STX Plates 24 hours \pm 2 hours at 37°C \pm 1°C. Incubation of 3M Petrifilm STX Disks at 37°C \pm 1°C for 3 hours.

Interpretation

Calculate the number of microorganisms present in the test sample according to ISO 7218² for one 3M Petrifilm STX Plate per dilution. Counting range is:

- Less than or equal to 150 red-violet colonies and/or less than or equal to 300 total colonies.
- Less than or equal to 150 pink zones.

Read the 3M Petrifilm STX Plates after 3 hours of incubation time is complete.



3M 01/9-04/03A, 01/9-04/03B
ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS
<http://nf-validation.afnor.org/en>

For more information about end of validity, please refer to NF VALIDATION certificate available on the web-site mentioned above.

References

1. U.S. Food and Drug Administration. Code of Federal Regulations, Title 21, Part 58. Good Laboratory Practice for Nonclinical Laboratory Practice Studies.
2. ISO 7218. Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations.
3. ISO/IEC 17025. General requirements for the competence of testing and calibration laboratories.
4. U.S. Food and Drug Administration. 1998. Bacteriological Analytical Manual, 8th ed., (Revision A), Appendix 3.64.
5. ISO 6887. Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination,
6. ISO 16140. Microbiology of food and animal feeding stuffs –Protocol for the validation of alternative methods.
7. ISO 6888-1. Microbiology of food and animal feeding stuffs –Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species), Part 1: Technique using Baird-Parker agar medium.
8. ISO 6888-2. Microbiology of food and animal feeding stuffs –Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species), Part 2: Technique using rabbit plasma fibrinogen agar medium.

Explanation of Symbols

www.3M.com/foodsafety/symbols

AOAC is a registered trademark of AOAC INTERNATIONAL
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