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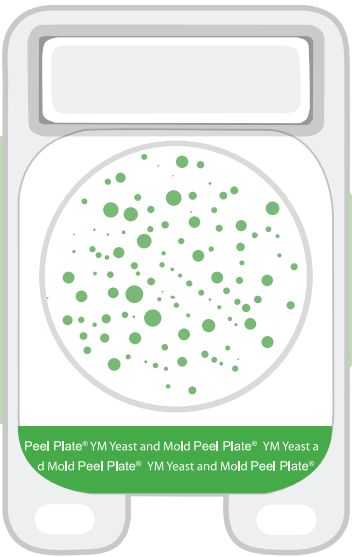

OPERATOR'S MANUAL

PeelPlate® YM HV

YEAST AND MOLD HIGH VOLUME

FOR DETECTION AND ENUMERATION OF YEAST AND MOLD

Contents

Kit Information

Introduction

Peel Plate® Yeast and Mold High Volume (YM HV) test detects and enumerates yeast and mold microorganisms in 5 mL sample volumes. The method is applicable for detection of fungi in samples when incubated at 25 ± 3 °C in the dark. To run a test, a 5 mL sample or sample dilution is added to the plate and incubated for 3 to 5 days. Yeast and mold colonies will appear as green, blue, or dark brown round or filamentous shapes. Molds may also produce natural pigments. Peel Plate YM HV 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 limit of detection is 1 or greater colony forming units per 5 milliliter or gram (CFU/ 5 mL or 5g) of test sample. The accurate quantitative range for yeast and mold is 10 to 150 CFU/plate; however, if substantial amounts of mold are present, depending on the type of mold, the upper countable limit may have to be lowered at the discretion of the analyst.

Kit Contents, Storage, and Testing Conditions

A test kit (item code PP-YMHV-25K) contains 25 tests in a desiccated foil bag containing a blue indicator desiccant. Item Code PP-YMHV-100K contains 4 bags of 25 tests.

Kits are not required to be shipped refrigerated.

Store kits in foil bag in refrigerator* or in ambient temperature (15 to 30 °C) for up to 1 month.

Open foil bag and remove the number of plates needed for analysis. Perform testing in a clean, dry testing area at ambient temperature. 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 and is required for US Certified Labs**

Principle

Peel Plate YM HV media is based on potato dextrose medium with selective agents to support and colorimetrically identify the growth of yeast and mold in test samples. The Peel Plate YM HV test contains enzyme substrates that turn blue when detecting phosphatase and glucosidase enzymes that are produced by growing fungi. The Peel Plate YM HV test also contains gelling and wicking agents which absorb and diffuse the sample.

Applicability

The Peel Plate YM method has been validated for detection of yeast and mold in dairy products (liquid, solid and cultured), juices, and bakery extracts and found not significantly different from reference method FDA-BAM, Chapter 18. The method has also been validated to detect fungi from environmental surface sponges of food contact surfaces. Samples should be 10-fold serially diluted into the countable range of 10 to 150 CFU/plate. The Peel Plate YM method has been applied to the larger volume HV plates.

Peel Plate YM HV tests have been evaluated in the claimed foods with 0.1 % peptone water, but have not been evaluated with all possible food products, food processes, testing protocols or with all possible microorganism strains.

Precautions:

- Observe Good Laboratory Practices for microbial testing. Avoid specimen contamination.
- Perform tests with clean, washed and gloved hands assuming potential pathogenic bacteria.
- Test on a level surface in a clean area, free of dust and blowing air.
- Avoid hand contact with test samples and the Peel Plate YM HV medium.
- After plating, replace adhesive cover so it lies flat with no wrinkles to avoid drying out the rehydrated medium during incubation.

Sample Preparation

Liquid Dairy	• White milk dairy samples (raw milk and pasteurized whole, lower fat %, and skim) may be tested neat or serially diluted to a countable range (10 to 150 CFU/plate). o To serially dilute, add 25 mL into 225 mL 0.1% peptone water or microbiologically suitable dilution buffer. Other automated dilution pipets and dilution schemes are acceptable.
Solid Dairy	• Add 25 g of solid dairy (ice cream, sour cream, heavy cream, etc.) to 225 mL of 0.1% peptone water or microbiologically suitable dilution buffer to reach countable range (10 to 150 CFU/plate). • For milk powders and evaporated/condensed, reconstitute with water to normal milk solid content and let any undissolved solids settle. Test liquid fraction as Liquid Dairy.
Bakery	• Add 25 g of flour, or baked products to 225 mL of 0.1 % peptone water or microbiologically suitable dilution buffer, homogenize for 2 minutes, and let settle to extract sample. • Continue to dilute 10 mL of prior dilution in 90 mL of dilution blank to reach countable range (10 to 150 CFU/plate).
Fruit Juices	• Add 5 mL of juice directly. Raw citrus juice may require pH adjustment. Clear or filtered juices and dilutions of citrus should not require a pH adjustment. • Continue to dilute 10 mL of prior dilution in 90 mL of 0.1 % peptone water or dilution buffer to reach countable range per plate (10 to 150 CFU/plate).
Environmental Swab	• Refer to Peel Plate Sample Preparation Addendum.

Test Procedure

Step 1

- Label plate on clear side using marker or bar code strip. Do not mark or label the uplifted 75 mm circular area.

Step 2

- For best results, hold plates at room temperature prior to plating.
- Invert and apply pressure with fingers to the back as shown and lift tab.
- Pull the adhesive cover completely 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 5.0 mL of sample or sample dilution to the center of disc. Expel within 2 to 3 seconds 1-2 cm above surface.

Step 4

- Sample will diffuse towards the edges of plate. For viscous samples lift plate and rotate to ensure proper distribution of sample.
- Re-apply the cover and smooth around the edges of the plate to seal the adhesive; avoid wrinkling.
- Allow plate to sit undisturbed 30 seconds before moving.

Step 5

- Incubate plates with clear side up, as shown.
  - Incubate in the dark at room temperature or at a controlled temperature, 25 ± 3 °C for 3 to 5 days
  - Plates can stack by aligning the feet and rectangle platform. Stacking plates up to 12 high will not affect plate heat transfer.

Analysis of Results

- At the end of the incubation period, observe plates for colonies through the clear side of the Peel Plate YM test. Each blue or blue/grey/green/brown spot represents one CFU. The sum of spots is reported as the total yeast and mold CFU/5 mL of the diluted sample.
- Multiply CFU/5 mL by dilution and then divide by 5 to calculate CFU/(mL or g) of original sample.
- In case of spreading fungi, score one CFU for each defined spot. Blended or spreading colonies are scored as a single CFU.
- Counts of 10 to 150 CFU/plate are considered countable, while counts outside that range are considered estimates.

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 5.0 mL of sterile dilution buffer to verify no detectable yeast or mold after incubation.
- Negative Control: Prepare Negative Control by autoclaving the appropriate dilution of test sample at 121 °C for 15 minutes. Cool to 4 °C and test 5.0 mL. Verify no detectable yeast or mold in the Negative Control.
- Positive Control: Spike a sample with known yeast or mold culture or a combination. Dilute sample to countable range of 10 to 150 CFU/mL and test 5.0 mL to verify detection after incubation.

Disposal

Microbiological cultures and reagents should be collected into biohazard bags and autoclaved. Dispose according to local, state, and federal regulations.

Technical Support

For questions contact a local representative or Charm Sciences at +1.978.687.9200 or support@charm.com.

Order Information

Description	Quantity	Kit Code
Peel Plate YM HV Tests	25	PP-YMHV-25K
	100	PP-YMHV-100K

Peel Plate HV tests for E. coli/coliforms, and coliform are also available. Peel Plate 1 mL sample tests for aerobic bacteria, E. coli/coliforms, coliform, yeast and mold, enterobacteriaceae 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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