hygiena

BAX® System PCR Assay for

Yeast and Mold

Part KIT2015 (D12522091)

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 010902

KIT CONTENTS

96 PCR tubes with tablets (1 bag of 12 x 8 strips)
96 flat optical caps (12 x 8 strips)
1 bottle of protease (400 µL)
2 bottles of YM lysis buffer (12 mL)

SUPPLEMENT KIT CONTENTS (D12522119)

96 Yeast and Mold Disrupter tubes
2 bottles of DNA Stabilizer (1 mL)

INTENDED USE

Yeasts and molds are a major cause of food spoilage that can cause food products to look, smell or taste bad when present at high levels. Food processors and associated laboratories can use the BAX® System as a quick and reliable method for detecting Yeast and Mold at various cutoff threshold (action levels) concentrations in selected foods that are the basis for product-release decisions. This assay is designed to report yes/no results for yeast and mold in food products after 44 hours of enrichment at a variety of action levels. In addition, a direct testing protocol for non-enriched samples can be used for labs that accept a relatively high level of target (≥ 500 cfu/g) and want to ship the same day. With a processing time of approximately 3.75 hours in the BAX® System Q7 instrument, the method returns results comparable to culture methods, but with a significantly faster time to result.

BAX® Systems are designed for use by qualified lab personnel who follow standard microbiology laboratory practice, including the safe handling and disposal of potentially pathogenic materials.

***Note**: Please refer to your accreditation agency for any specific requirements.*

Field of use: Data obtained from the BAX® System should not be used for human diagnostic or human treatment purposes. Equipment is not approved by the United States Food and Drug Administration or any other U.S or non-U.S. regulatory agency for use in human diagnostics or treatment. The BAX® System should not be used as the sole basis for assessing the safety of products for release to consumers. The information generated is only to be used in conjunction with the user's regular quality assurance program. Not approved for clinical diagnosis. Use for research and development, quality assurance and quality control under supervision of technically qualified persons.

PRINCIPLE OF THE METHOD

See the BAX® System User Guide for an overview of how the BAX® System method uses automated, Polymerase Chain Reaction (PCR) technology.

MATERIALS

BAX® System PCR Assay for Yeast and Mold (Part KIT2015 [D12522091])

BAX® System Yeast and Mold Supplement kit (Part KIT2014 [D12522119])

BAX® System start-up package (equipment and supplies for up to 192 tests)

- BAX® System cycler/detector and computer work station
- Heating blocks with inserts* capable of maintaining 37±2ºC and 95±3ºC
- Cooling blocks with inserts*
- PCR tube holder
- Capping/decapping tools
- Adjustable mechanical pipettes (5-50 µL; 20-200 µL)
- Repeating pipette
- Multi-channel pipette (8 channels – 5-50 µL)
- Cluster tubes with caps and racks
- Pipette tips with barriers
- Powder-free nitrile gloves

**The Automated Thermal Block (Catalog No. MCH2023 [D14614252]) may be used in place of heating and cooling blocks.*

Stomacher with bags

Incubator capable of maintaining directed enrichment temperatures within ±2°C

Cell disrupter device – Disrupter Genie (VWR Catalog No. 14216-180)

Standard Solutions (such as Butterfield's phosphate buffer, 0.1% peptone buffer or other appropriate buffer for diluting samples)

STORAGE AND SHELF LIFE

- Reagent packages should be kept refrigerated at 2–8°C. Do not freeze.
- Reagents should be used by the expiration date stamped on the individual labels.
- After protease has been added to the YM lysis buffer, shelf life of the solution is 2 weeks when stored at 2-8°C.

PRECAUTIONS

The BAX® System method includes sample enrichment procedures that nourish the growth of potential pathogens to detectable levels. Because pathogens can cause human illness, appropriate safety precautions must be taken when handling samples, media, reagents, glassware and other supplies and equipment that could be contaminated with potentially pathogenic bacteria.

Reagents used with the BAX® System assays should pose no hazards when used as directed. Before using this assay, please review the Safety Data Sheets (SDS) included with your BAX® System purchase and also available at www.hygiena.com. Refer to your site practices for safe handling of materials at extreme temperatures.

SOFTWARE REQUIREMENTS

Before using this assay for the first time, install the most current version of the BAX® System software, then run a calibration report to check that "Yeast and Mold Direct" and "Yeast and Mold Enriched" appear in the list of calibration files. See "Troubleshooting Calibration" in the BAX® System User Guide for details.

If the report list does not contain "Yeast and Mold Direct" and "Yeast and Mold Enriched" must recalibrate the Q7 instrument to load the required dyes. Be sure to allow enough time to complete the calibration (about 1.5 to 2 hours) before starting the assay. For instructions and tips on calibrating the instrument, see the BAX® System User Guide.

SAMPLE PREPARATION

1. Homogenize and Enrich Samples

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- *Yogurt, corn starch and milk-based powdered infant formula:* Homogenize 25 g sample with 225 mL 0.1% peptone water. Transfer sample in triplicate from stomacher bag to disruptor tubes. The amount transferred to disrupter tubes is determined by action level (see action level table in the BAX® System User Guide). Incubate all disruptor tubes for 44 hours at 25°C.

TEST PROTOCOL

2. Prepare Equipment

2.1 Turn on the heating blocks for 37ºC and 95ºC*.
2.2 Make sure cooling blocks are chilled to 2-8ºC*.
**If using the Automated Thermal Block, follow the instructions in the Automated Thermal Block User Guide for running the Gram Negative program.*
2.3 Power on the Q7 instrument and launch the BAX® System application.
2.4 Create a rack file (see User Guide for details).
***Note**: You have two choices when testing for yeast and Mold. Select Yeast and Mold Enriched for samples that have been enriched. Select Yeast and Mold Direct for samples that have not been enriched.*
***Optional:** When selecting the Yeast and Mold Direct protocol, the ratio threshold for samples can be manually entered to set the sensitivity limit for the assay.*

3 Disrupt Samples

3.1 Add 20 µL DNA stabilizer to each disruptor tube.
3.2 Place tubes in disruptor device and agitate for 15 minutes.
3.3 Return tubes to the rack and repeat with the remaining disruptor tubes until all samples have been disrupted.

4 Perform Lysis

4.1 Break cluster tubes apart.
4.2 Label and arrange cluster tubes in rack according to the rack file.
4.3 Prepare lysis reagent by adding 150 µL protease to one 12 mL bottle of YM lysis buffer.
4.4 Transfer 200 µL lysis reagent to each cluster tube.
4.5 Transfer 7 µL disrupted sample from each triplicate disruptor tube to one cluster tube.
4.6 Heat at 37°C for 20 minutes.
4.7 Heat at 95°C for 10 minutes.
4.8 Cool at 2-8°C for 5 minutes.

5. Hydrate PCR Tablets

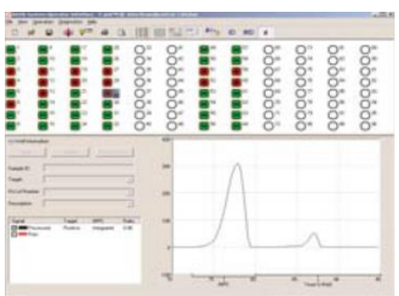
5.1 Initialize the instrument by selecting RUN FULL PROCESS from the OPERATION menu.
5.2 Place a PCR tube rack onto a chilled (2-8°C) PCR cooling block.
5.3 Arrange strips of PCR tubes according to your rack file.
5.4 Remove the caps from the first strip of tubes with the decapping tool.
5.5 Transfer 50 µL lysate (from step 4.8) into PCR tubes, then seal with flat optical caps.
5.6 Repeat with remaining strips of PCR tubes until all PCR tablets have been hydrated.

6. Amplify and Detect

6.1 At the "Ready for Rack Load" prompt, click the NEXT button and open the instrument drawer.
6.2 Place the rack of PCR tubes over the wells in the drawer, and check that the tubes are seated correctly.
6.3 Close the drawer, and click the NEXT button to begin automated processing.

7. Review Results

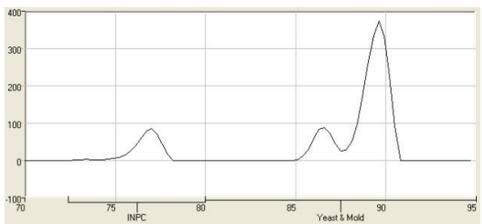
Qualitative results are based on the action level that you select during sample preparation. Results are displayed as a grid of color-cued icons in the top half of the screen:



⊖	Green (-)	=	Negative for target organism
⊕	Red (+)	=	Positive for target organism
?	Yellow (?)	=	Indeterminate result*
?	Yellow (?) with red slash	=	Signal error*

*Refer to the troubleshooting section in the User Guide for assistance.

- Positive results reflect the presence of yeast and mold at or above the desired action level. Positive reactions display one peak in the positive control range (74-81°C) and one or two peaks in the target range (81-90°C).



Strong Yeast and Mold positive

- Negative results indicate that any yeast and mold in the sample is below the desired action level. Negative reactions display only the positive control peak.

CONFIRMATION

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If desired, BAX® System results can be confirmed from the reference culture method appropriate for the sample type, such as:

- U.S. FDA Bacteriological Analytical Manual (BAM)
- USDA FSIS Microbiology Laboratory Guidebook (MLG)
- Health Canada Compendium of Analytical Methods
- International Organization for Standardization (ISO)

DISPOSAL

Decontaminate materials and dispose of biohazardous waste per your site practices and as required by federal, state and local regulations.

VALIDATION

The BAX® System PCR Assay for Yeast and Mold has been certified by the AOAC Research Institute as Performance Tested℠ Method #010902. This test kit's performance was reviewed by AOAC-RI and found to perform to the manufacturer's specifications. Validation studies for select foods demonstrated BAX® System sensitivity and specificity equal to or better than the reference culture based methods.

TECHNICAL ASSISTANCE

For questions or comments, please contact your local distributor. You can also call 800-863-6842 in the U.S., 1-302-695-5300 outside the U.S., or email diagnostics.support@hygiena.com.

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4. The accuracy of the BAX® System can be affected by factors over which Hygiena has no control, including, without limitation, the use of the Equipment, assays and/or media in a manner that is contrary to the conditions of use, the procedures or the instructions specified by Hygiena. Because of the large number of factors over which Hygiena has no control, Hygiena makes no promise or guarantee of the accuracy of or results obtained from the use of the BAX® System. In particular, Hygiena disclaims any warranty or liability and assumes no responsibility whatever for the failure of the BAX® System due, in whole or in part, to user's failure to: (a) properly maintain Equipment, (b) maintain specified operating or storage conditions, (c) follow the specified instructions, or (d) use the proper microbiological techniques consistent with the standard of care accepted in the industry for the proper collection, storage, handling and preparation of the sample.

5. Externally caused failures, such as improper sample preparation, improper storage or loading of reagents, electrical outages, or out-of-specification environmental conditions are not covered under this warranty. Equipment failures caused by spills, abuse, misuse, negligence, or improper operation are not covered by this warranty. Modifications, service or repairs by parties other than Hygiena-authorized providers are not covered by this warranty and, in fact, void this warranty. Circumstances beyond the reasonable control of Hygiena, including fire, explosions, accidents, flood, labor trouble or shortage, war, act of or authorized by any government, inability to obtain suitable material, Equipment, fuel, power or transportation, or acts of God are not covered under this warranty.

6. The BAX® System is designed to test only for the presence of the target organisms specified in the particular assay. The BAX® System has been tested against many, but not all, strains of the target within the sample types specified in the user documentation. Hygiena, therefore, cannot and does not make any representation or warranty that the BAX® System is capable of detecting every organism in the target genus, serotype, or species in any sample source. Accordingly, the BAX® System should not be used as the sole test for the release of user's products, nor should it be used as the sole basis for determining the safety of user's products.

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