

Instructions for Use

RAPID TREHALOSE BROTH

Cat. no. Z205	Rapid Trehalose Broth, 13x100mm Tube, 0.35ml	20 tubes/box
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INTENDED USE

Hardy Diagnostics Rapid Trehalose Broth is a rapid screening method to aid in the detection of *Candida (Torulopsis) glabrata*.

SUMMARY

Within the genus *Candida*, *C. albicans* is the most frequently isolated as the cause of disease in clinical labs. However, the need to screen for *Candida glabrata* has risen in importance as it has been isolated as the cause of major infections, particularly in immunosuppressed patients.

Hardy Diagnostics Rapid Trehalose Broth is a rapid screening method for *C. glabrata* on the basis of rapid trehalose assimilation at 42°C. *Candida glabrata* is capable of utilizing trehalose more quickly than other *Candida* species. The elevated incubation temperature increases the specificity of the test.

Rapid Trehalose Medium is derived from the formula described by Stockman and Roberts.⁽⁶⁾ It utilizes yeast nitrogen base as a source of nitrogenous compounds. Yeast nitrogen base is low in other carbohydrates that might interfere with the rapid test. Bromcresol green is the indicator, allowing the visualization of an acid shift that is indicative of a positive reaction. Trehalose is incorporated into the medium at a high concentration as the carbon source. This product is best used in conjunction with HardyCHROM™ *Candida* (Cat. no. G301). *C. glabrata* will appear as smooth pink colonies, often with a darker pink or mauve center, on this screening method and should be confirmed with the trehalose test.

FORMULA

Ingredients per liter of deionized water:*

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Trehalose	40.0gm
Yeast Nitrogen Base	10.7gm
Bromcresol Green	20.0mg

Final pH 5.4 +/- 0.1 at 25°C.

* Adjusted and/or supplemented as required to meet performance criteria.

STORAGE AND SHELF LIFE

Storage: Upon receipt store media at 2-8°C. away from direct light. Media should not be used if there are any signs of deterioration, discoloration, contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "[Storage](#)" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "[Guidelines for Isolation Precautions](#)" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections*.

Sterilize all biohazard waste before disposal.

Refer to the document "[Precautions When Using Media](#)" for more information.

PROCEDURE

Specimen Collection: This product is not intended for the primary isolation of patient specimens. This product is intended to be used in conjunction with other biochemical and/or serologic tests to identify cultures of isolated organisms.

1. Obtain isolated colonies that are 24-48 hours old, preferably from HardyCHROM™ Candida (Cat. no. G301).
2. Inoculate the Rapid Trehalose Broth with the yeast isolate, creating a suspension at least as dense as a 1.0 McFarland Standard.
3. Incubate the test at 42°C. for three hours.
4. Observe for a color change from blue to yellow. Do not read the tubes after three hours.

INTERPRETATION OF RESULTS

C. glabrata appear microscopically as tiny budding yeast cells with no hyphae, pseudohyphae, blastospores or chlamydospores. *C. glabrata* colonies will appear as smooth pink, often with darker pink to mauve centers, on HardyCHROM™ Candida (Cat. no. G301).

A positive Rapid Trehalose Broth test is indicated by a color change from blue to yellow within three hours. This is indicative of *C. glabrata*.

Negative tests in Rapid Trehalose Broth remain green-yellow, green, or blue. These reactions are indicative of yeast isolates other than *C. glabrata*.

Typical reactions of common *Candida* species.^{(1)*}

Organism	Rapid Trehalose	Germ Tube	Morphology on Corn Meal Agar with Tween®			Color on HardyCHROM™ Candida (Cat. no. G301)
			Pseudo- or true-hyphae	Blasto-conidia	Chlamydo-spores	
<i>C. glabrata</i>	+	-	-	-	-	Light pink to pink, with a darker pink or mauve center
<i>C. albicans</i>	-	+	+	+	+	Emerald green to metallic green
<i>C. tropicalis</i>	-	-	+	+	-	Dark blue to metallic blue with blue halos

* Some strain to strain variation may occur, and isolates may require more biochemical and/or serological tests for complete identification. Refer to the listed references for more information regarding the identification and differentiation of *Candida* species.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

This medium is intended to be used a screening medium for *C. glabrata*. Rare clinical strains of *C. glabrata* have demonstrated weak reactions with Rapid Trehalose media. Other biochemical and/or serological tests may be required for complete identification of this organism.

Rare *Candida tropicalis* strains may give a false-positive reaction in Rapid Trehalose Broth.

Do not incubate longer than 3 hours. Extended incubation times may lead to erroneous results.⁽⁷⁾

A heavy inoculum must be used. The turbidity of the Rapid Trehalose Broth should be at least equivalent to a 1.0 McFarland Standard after inoculation. Tubes that are inoculated too lightly may give false-negative reactions.⁽⁷⁾

Refer to the document "[Limitations of Procedures and Warranty](#)" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, slides, staining supplies, other culture media, microscopes, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	

<i>Candida glabrata</i> ATCC® 66032	*	3hr	42°C	Aerobic	Positive; media becomes yellow
<i>Candida albicans</i> ATCC® 60193	*	3hr	42°C	Aerobic	Negative; media remains blue
<i>Candida tropicalis</i> ATCC® 750	*	3hr	42°C	Aerobic	Negative; media remains blue

* Refer to the document "[Inoculation Procedures for Media QC](#)" for more information.

USER QUALITY CONTROL

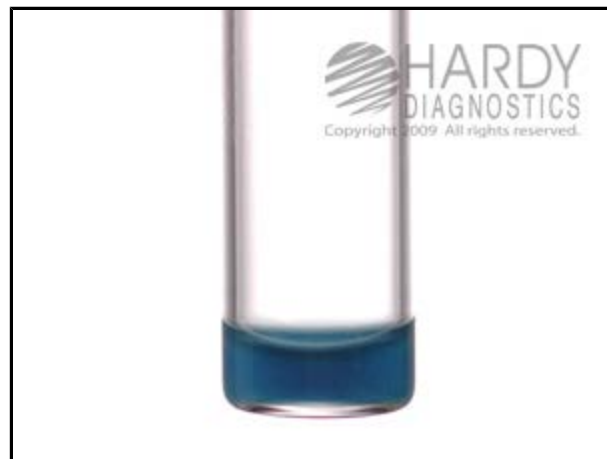
End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics [Certificate of Analysis](#) website. Also refer to the document "[Finished Product Quality Control Procedures](#)," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

Rapid Trehalose Broth should appear clear, and dark blue in color.



Candida glabrata (ATCC® 66032) growing in Rapid Trehalose Broth (Cat. no. Z205). The yellow color change within three hours was indicative of a positive trehalose reaction. Incubated aerobically for three hours at 42°C.



Candida albicans (ATCC® 60193) growing in Rapid Trehalose Broth (Cat. no. Z205). No yellow color change within three hours was indicative of a negative trehalose reaction. Incubated aerobically for three hours at 42°C.

REFERENCES

1. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.
2. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.
3. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.
4. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.
5. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory

Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

6. Stockman, L. and G. Roberts. 1985. Abstracts of Annual Meeting of American Society of Microbiology, p.377 (F-80). Washington, D.C.

7. Hardy Diagnostics. 1998. Internal testing results.

ATCC is a registered trademark of the American Type Culture Collection.

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