IFU

Instructions for Use

CRITERION™ ROSE BENGAL AGAR BASE

Cat. no. C8100	CRITERION™ Rose Bengal Agar Base	64gm
Cat. no. C8101	CRITERION™ Rose Bengal Agar Base	500gm
Cat. no. C8102	CRITERION™ Rose Bengal Agar Base	2kg
Cat. no. C8103	CRITERION™ Rose Bengal Agar Base	10kg
Cat. no. C8104	CRITERION™ Rose Bengal Agar Base	50kg

INTENDED USE

Hardy Diagnostics CRITERION™ Rose Bengal Agar Base is intended for the selective isolation and enumeration of fungi from environmental and food sources. Chloramphenicol may be added aseptically to the basal medium for increased selectivity.

This dehydrated culture medium is a raw material intended to be used in the making of prepared media products, which will require further processing, additional ingredients, or supplements.

SUMMARY

The large, diverse group of yeasts and molds, known as Fungi, consists of several hundred species. Fungi are present in, and recovered from, air, soil, lakes, ponds, rivers, streams, wastewater, and well waters.[5] Due to their heterotrophic nature, and their ability to adapt to a wide range of environmental conditions, fungi are also frequently encountered as contaminants in various commodities including foods, inadequately cleaned food processing equipment, and food storage facilities. Since yeasts and molds can grow over wide pH and temperature ranges, growth can occur on almost any type of food including processed foods and food ingredients.[3,6]

Traditionally, low pH media have been used to enumerate yeasts and molds from water, soil, and food. Such media are now believed to be inferior to antibiotic supplemented media. The use of antibiotics, rather than acid, for suppressing bacteria results in improved recovery of injured (acid-sensitive) fungal cells, better control of bacteria, and less interference during counting from precipitated food particles.[4] CRITERION™ Rose Bengal Agar Base contains chloramphenicol, a broad spectrum antibiotic, which can be added as a selective agent to inhibit bacterial growth.[3]

In addition to chloramphenicol, rose bengal is added to the media, to increase the selectivity and help control overgrowth by rapidly growing molds such as *Neurospora* and *Rhizopus* species. Besides providing better isolation of slow growing fungi, rose bengal dye is also taken up my fungal isolates, thereby aiding in their recognition. Smith and Dawson found that rose bengal added to a near-neutral medium (pH of 6.8), allowed for more colonies to develop than did an acidified medium (pH of 4.2).[8] CRITERION™ Rose Bengal Agar Base also contains soy peptone as a source of carbon and nitrogen, dextrose as an energy source, and magnesium sulfate to provide trace elements.[2,8]

FORMULA*

Gram weight per liter:	32.0gm/L
Dextrose	10.0gm
Papaic Digest of Soybean Meal	5.0gm
Monopotassium Phosphate	1.0gm
Magnesium Sulfate	0.5gm
Rose Bengal	0.05gm
Agar	15.0gm

Final pH 7.2 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Store the sealed bottle(s) containing dehydrated culture medium at 2-30ºC. Dehydrated culture medium is very hygroscopic. Keep lid tightly sealed. Protect dehydrated culture media from moisture and light. The dehydrated culture media should be discarded if it is not free-flowing or if the color has changed from its original beige to pale pink.

Store the prepared culture media at 2-8ºC.

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 blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory 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.

METHOD OF PREPARATION FOR DEHYDRATED CULTURE MEDIA

1. Suspend 32.0gm of the dehydrated culture media in 1 liter of distilled or deionized water. Stir to mix thoroughly.

2. Heat to boiling to dissolve completely. Do not overheat.

3. Add 100.0mg of chloramphenicol to media and mix well.

4. Sterilize in the autoclave at 121ºC. for 15 minutes.

5. Cool to 45-50ºC.

PROCEDURE AND INTERPRETATION OF RESULTS

For information on procedures and interpretation of results, consult listed references or refer to the prepared media Instructions for Use (IFU) for Cat. No. W87.

LIMITATIONS

Although this medium is selective for fungi, microscopic examination is recommended for presumptive identification.

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

Some formulations may require a settling period before pH testing of the prepared medium. If the pH is tested immediately after preparation and is out of specification, retest the medium after 24 hours to obtain final pH results. Always take pH reading at room temperature.

It is important to not expose this medium to light since photodegradation of rose bengal produces compounds that are toxic to fungi.[3]

Chloramphenicol may not be sufficient to inhibit all bacterial flora.[2]

As fungal colonies take up the rose bengal dye, it may be necessary to subculture onto a secondary medium prior to inoculation onto Rose Bengal Agar.[2]

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as autoclaves, incinerators, and incubators, etc., are not provided. Chloramphenicol must be purchased separately, as it is not included in the Rose Bengal Agar Base formula.

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	
CRITERION™ Rose Bengal Agar Base with Chloramphenicol Supplement:					
Candida albicans ATCC® 10231	A	48-96hr	15-30°C	Aerobic	Growth; pink smooth raised colonies
Aspergillus brasiliensis ATCC® 16404	A	3-5 days	15-30°C	Aerobic	Growth; white and filamentous, black specks on colonies
Escherichia coli					

ATCC® 25922	B	24hr	35°C	Aerobic	Inhibited

* Refer to the document "Inoculation Procedures for Media QC" for more information.

USER QUALITY CONTROL

Users of dehydrated 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. In addition, refer to the following document "Finished Product Quality Control Procedures," for more information on QC or see the reference(s) for more specific information.

PHYSICAL APPEARANCE

CRITERION™ Rose Bengal Agar Base powder should appear homogeneous, free-flowing, and beige to pale pink in color. The prepared media should appear slightly opalescent, and bright pink in color.

REFERENCES

1. Atlas, R.M. 1997. *Handbook of Microbiological Media*, 2nd ed. CRC Press, Inc., Boca Raton, FL.

2. MacFaddin, J.F. 1985. *Media for Isolation, Cultivation, Identification, Maintenance of Bacteria*, Vol. I. Williams & Wilkins, Baltimore, MD.

3. APHA Technical Committee on Microbiological Methods for Foods. *Compendium of Methods for the Microbiological Examination of Foods*, APHA, Washington, D.C.

4. American Public Health Association. *Standard Methods for the Examination of Dairy Products*, APHA, Washington, D.C.

5. American Public Health Association. *Standard Methods for the Examination of Water and Wastewater*, APHA, Washington, D.C.

6. U.S. Food and Drug Administration. *Bacteriological Analytical Manual.* AOAC, Arlington, VA. http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm.

7. Waksman, S.A. 1922. A method for counting the number of fungi in the soil. *J. Bacteriol.*; 7:339-341.

8. Smith, N.R., V.T. Dawson. 1944. The bacteriostatic action of Rose Bengal in media used for plate counts of soil fungi. *Soil Sci.*; 58: 467-471.

9. Cooke, W.B. 1954. The use of antibiotics in media for the isolation of fungi from polluted water. *Antibiotics and Chemotherapy*; 4:657-662.

10. Papavizas, G.C., C.B. Davey. 1959. Evaluation of various media and antimicrobial agents for isolation of soil fungi. *Soil Sci.*; 88:112-117.

11. Jarvis, B. 1973. Comparison of an improved Rose Bengal-Chlortetracycline Agar with other media for the selective isolation and enumeration of moulds and yeasts in foods. *J. Appl. Bact.* ; 36:723-727.

IFU-10245[A]

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

HDQA 2207B [D]