DICHLORAN ROSE BENGAL CHLORAMPHENICOL (DRBC) AGAR

Cat. no. G389	Dichloran Rose Bengal Chloramphenicol (DRBC) Agar, 15x100mm Plate, 18ml	10 plates/bag
Cat. no. W89	Dichloran Rose Bengal Chloramphenicol (DRBC) Agar, 15x100mm Plate, 26ml	10 plates/bag

INTENDED USE

Hardy Diagnostics Dichloran Rose Bengal Chloramphenicol (DRBC) Agar is recommended for the enumeration of yeasts and molds in food and dietary supplements.

This product is not intended to be used for the diagnosis of human disease.

SUMMARY

Fungi are recovered from air, soil, lakes, ponds, rivers, streams, wastewaters, and well waters. 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 initiate growth over a wide pH and temperature range, growth can occur on almost any type of food, including processed foods and food ingredients. [1,3]

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 such as Sabouraud Dextrose Agar. [5] Traditionally, low pH media are used to enumerate yeasts and molds from water, soil, and food. Such media are now believed to be inferior to selective media with antibiotics. The use of antibiotics for suppressing bacteria, rather than acid, results in improved recovery of injured (acid-sensitive) fungal cells, better control of bacteria, and less interference during counting from precipitated food particles. [2,6]

Hardy Diagnostics DRBC Agar contains peptone as a source of carbon and nitrogen, dextrose as an energy source, and magnesium sulfate to provide trace elements. [5] The medium contains chloramphenicol, which is added to inhibit most bacterial growth. [1] In addition to chloramphenicol, rose bengal is added to increase the selectivity and to help control overgrowth of rapidly growing molds such as *Neurospora* and *Rhizopus* species. Dichloran is added to the media to ihibit the spreading of molds by reducing colony diameters. [7] DRBC Agar conforms to the APHA guidelines for the mycological examination of foods. [1]

FORMULA

Ingredients per liter of deionized water:*

Dextrose	10.0gm

Peptone	5.0gm
Monopotassium Phosphate	1.0gm
Magnesium Sulfate	0.5gm
Chloramphenicol	0.1gm
Rose Bengal	0.025gm
Dichloran	0.002gm
Agar	15.0gm

Final pH 5.6 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8ºC. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), hemolysis - for media w/blood, contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration dating 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 quality control incubation times.

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." The "Guidelines for Isolation Precautions" is available from the Centers for Disease Control and Prevention at www.cdc.gov/ncidod/dhqp/gl_isolation.html.

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 M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

Refer to the document SDS Search instructions on the Hardy Diagnostics' website for more information.

PROCEDURE

1. If using the dilution method, add 40ml of the food sample to 200ml of 0.1% Peptone Water (Cat no. U201) and homogenize in a stomacher for 2 minutes. [3]

2. Inoculate 0.1ml of sample onto the agar surface.

3. Spread the inoculum evenly over the entire surface using a sterile bent glass rod or disposable spreader (Cat No. 174CS200).

4. Incubate plates at 22 to 25ºC. and examine plates after 3, 4, and 5 days of incubation. [1,3] Record results as colony forming units per gram of food.

INTERPRETATION OF RESULTS

Colonies should be apparent within five days of incubation. Yeast colonies will appear pink due to the uptake of rose bengal. Report counts as colony forming units (CFU) per gram or ml of sample.

LIMITATIONS

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

It is important to protect this medium from light since photodegradation of rose bengal produces compounds that are toxic to fungi. [1,4]

Due to the selective nature, some strains may grown poorly or fail to grow at all on this medium.

Chloramphenicol may not be sufficient to inhibit all bacterial flora.

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, disposable spreaders (Cat No. 174CS200), swabs, applicator sticks, other culture media, such as Peptone Water 0.1% (Cat No. U201), stomacher, 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 Certificates of Analysis (CofA). The following organisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Aspergillus brasiliensis ATCC® 16404	A	3-5 days	15-30°C	Aerobic	Growth; white and filamentous, black specks on colonies
Candida albicans ATCC® 10231	A	48-96hr	15-30°C	Aerobic	Growth; pink smooth raised colonies
Escherichia coli ATCC® 25922	B	24 hr	35°C	Aerobic	Inhibited
Bacillus subtilis ATCC® 6633	B	24 hr	35°C	Aerobic	Inhibited

* 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 certificates of analysis (CofA) available from Hardy Diagnostics Certificates of Analysis website. In addition, refer to the following document "Finished Product Quality Control Procedures," for more information on QC or see reference(s) for more specific information.

PHYSICAL APPEARANCE

Dichloran Rose Bengal Chloramphenicol (DRBC) Agar should appear slightly opalescent, and bright pink in color.

REFERENCES

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

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

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

4. Banks, J.G., R.G. Board. 1985. Preservation by the lactoperoxidase system (LP-S) of a contaminated infant formula. *Letters in Applied Microbiology.* 1:81–85.

5. 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.

6. King, Hocking and Pitt. 1979. *Appl. Environ. Microbiol.* 37:959.

7. Henson, OE. 1981. Dichloran as an Inhibitor of Mold Spreading in Fungal Plating Media: Effects on Colony Diameter and Enumeration. *Appl Environ Microbiol* . 42(4): 656–660.

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

HDQA 2207D [C]