IFU

Instructions for Use

ROSE BENGAL AGAR WITH CHLORAMPHENICOL

Cat. no. W87	Rose Bengal Agar with Chloramphenicol, 15x100mm Plate, 26ml	10 plates/bag
Cat. no. P42	Rose Bengal Agar with Chloramphenicol, Contact Plate, 15ml	10 plates/bag
Cat. no. U308	Rose Bengal Agar with Chloramphenicol, 16oz. Glass Bottle, 400ml	12 bottles/box
Cat. no. Q81	Rose Bengal Agar with 1.5X Chloramphenicol, 20x150mm Tube, 20ml Deep	100 tubes/box

INTENDED USE

Hardy Diagnostics Rose Bengal Agar with Chloramphenicol is recommended for the selective isolation and enumeration of fungi from environmental and food sources.

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.[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 initiate growth over a wide pH range 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] Hardy Diagnostics Rose Bengal Agar with Chloramphenicol contains the antibiotic chloramphenicol, which is added as a selective agent to inhibit most 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] Hardy Diagnostics Rose Bengal Agar with Chloramphenicol 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

Ingredients per liter of deionized water:*

Dextrose	10.0gm
Papaic Digest of Soybean Meal	5.0gm
Monopotassium Phosphate	1.0gm
Magnesium Sulfate	0.5gm
Chloramphenicol	0.1gm
Rose Bengal	0.05gm
Agar	15.0gm

Final pH 7.2 +/- 0.3 at 25ºC.

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

Note: Rose Bengal Agar with 1.5X Chloramphenicol (Cat. no. Q81) contains 0.15g Chloramphenicol.

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), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, 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 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.

PROCEDURE

For melting agar deeps: Liquefy the medium by autoclaving at 121°C for 1-3 minutes Cool the medium to 45-50°C and pour into sterile petri dishes. Allow the agar to solidify for at least 30 minutes prior to use. Alternatively, a covered,

boiling waterbath (100°C) can be used. There should be enough water in the waterbath to reach the top of the media line. Heat in a waterbath until melted through. A covered waterbath will help to reach and maintain the media temperature prior to dispensing.

Note: After autoclaving, do not heat media using a hot plate, heat block or waterbath for longer than 3 hours at 45-50°C. Melt only enough media that can be poured within a 3 hour time period. For optimal performance, sterile solidified medium should be remelted only once prior to use.

Yeasts and molds should be enumerated by a surface spread-plate technique rather than using pour plate methods. This technique provides maximal exposure of the cells to atmospheric oxygen, avoids heat stress from molten agar, gives more uniform growth and makes colony isolation easier. Agar spread plates should be dried overnight before being inoculated.[3,4,6] Pour plating should only be used when yeast or non-stressed mold cells are being detected.[4]

1. Inoculate 0.1ml of sample, or appropriate dilution, in duplicate onto the agar surface.

2. Spread the inoculum over the entire surface using a sterile bent glass rod, or disposable spreaders. Do not invert the plates.

3. Inoculated plates should be incubated undisturbed in an upright position at 22 to 30ºC for 7 days before colonies are counted. Room temperature incubation can be used if an incubator is not available.[3-5]

Interpretation of viable yeast and mold counts is often difficult as background data on expected and excessive levels for many foods have not been established. Determining the predominant species is also important.[3] Microscopic examination is recommended for presumptive identification. Biochemical testing using pure cultures is necessary for complete identification.

Plates with 15 to 150 fungal colonies are usually counted. If the mycoflora consists primarily of molds, the lower population range is selected; if primarily yeast colonies, the upper limit is counted. Report counts as colony forming units (CFU) per gram or ml of sample.[3-5] If counting must be delayed temporarily, plates can be held at 4°C for no longer than 24 hours.[5]

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 of bacteria and/or fungi.

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 loops, other culture media, swabs, applicator sticks, 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	
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
In addition to the above organisms, Cat. no. Q81 is tested with the following organism:					
Staphyloccus aureus ATCC® 6538	B	24hr	35°C	Aerobic	Inhibited

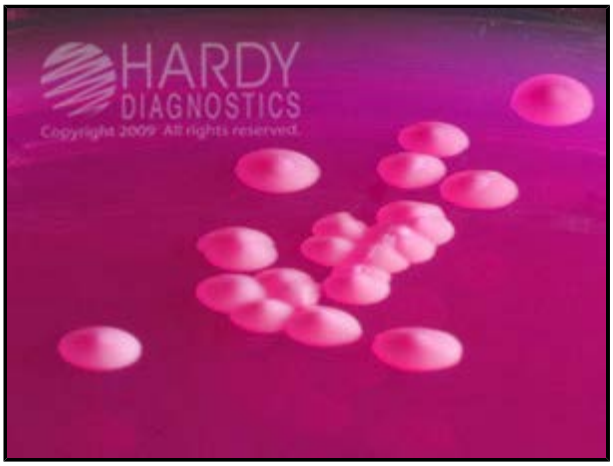
* Refer to the document "Inoculation Procedures for Media QC" for more information.

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 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

Rose Bengal Agar with Chloramphenicol should appear slightly opalescent, and bright pink in color.



Candida albicans (ATCC® 10231) colonies growing on Rose Bengal Agar with Chloramphenicol (Cat. no. W87). Incubated aerobically for 48 hours at 30ºC.

Aspergillus brasiliensis (ATCC® 16404) colonies growing on Rose Bengal Agar with Chloramphenicol (Cat. no. W87). Incubated aerobically for 72 hours at 30ºC.

REFERENCES

Uninoculated plate of Rose Bengal Agar with Chloramphenicol (Cat. no. W87).

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IFU-10731[A]

HARDY DIAGNOSTICS

1430 West McCoy Lane, Santa Maria, CA 93455, USA
Phone: (805) 346-2766 ext. 5658
Fax: (805) 346-2760
Website: HardyDiagnostics.com
Email: TechnicalServices@HardyDiagnostics.com
Ordering Information

Distribution Centers:
California · Washington · Utah · Arizona · Texas · Ohio · New York · Florida · North Carolina

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

HDQA 2207F [D]