IFU

Instructions for Use

BRILLIANT GREEN AGAR

Cat. no. G75	Brilliant Green Agar, 15x100mm Plate, 18ml	10 plates/bag

INTENDED USE

Hardy Diagnostics Brilliant Green Agar is recommended for the selective enrichment of *Salmonella* spp. other than *Salmonella* Typhi and *Salmonella* Paratyphi from clinical and non-clinical specimens.

SUMMARY

Kristensen, et al., in 1925, first described use of Brilliant Green Agar as a primary plating medium for the isolation of *Salmonella* spp.[9] Kauffmann modified the formula in 1935.[10]

The current formulation incorporates phenol red as the pH indicator and brilliant green as an inhibitory agent that acts against gram-positive organisms and gram-negative bacilli. Organisms that ferment lactose and/or sucrose exhibit yellow to yellow-green colonies surrounded by a yellow-green zone. *Salmonella* appears as red to pink-white colonies surrounded by a red zone in the medium.

Brilliant Green Agar is not recommended for the selective isolation of *Salmonella* Typhi.

FORMULA

Ingredients per liter of deionized water:*

Lactose	10.0gm
Sucrose	10.0gm
Sodium Chloride	5.0gm
Pancreatic Digest of Casein	5.0gm
Peptic Digest of Animal Tissue	5.0gm
Yeast Extract	3.0gm
Phenol Red	0.08gm
Brilliant Green	12.5mg
Agar	20.0gm

Final pH 6.9 +/- 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, 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 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: Consult listed references for information on specimen collection.[1-4,6] Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If there is to be a delay in processing, the specimen should be inoculated onto an appropriate transport medium (e.g. Cary-Blair) in order to maintain viability of the organisms.

Method of use: Allow medium to warm to room temperature prior to inoculation. Inoculate medium using a four-quadrant streak to produce colonies. A heavily inoculum should be used since the medium is quite inhibitory.[3] Incubate aerobically at 35ºC. for up to 48 hours.

It is recommended that other primary plating media (e.g. MacConkey Agar, SS Agar) or fluid enrichments (e.g. Tetrathionate Broth, Selenite Cystine Broth) be used in conjunction with Brilliant Green Agar to ensure recovery of gram-negative intestinal pathogens.

INTERPRETATION OF RESULTS

Typically, *Salmonella* spp. and other non-lactose-fermenters appear as red to pink-white colonies surrounded by brilliant red zones in the medium. Lactose-fermenting or sucrose-fermenting organisms appear as yellow to yellow-green colonies surrounded by yellow-green zones in the medium.

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.

The recovery of many *Salmonella* spp. is greatly jeopardized if stool specimens remain unpreserved for more than three hours before processing. If there is to be a delay in processing, the specimen should be inoculated into the appropriate transport medium in order to maintain viability of the organisms.

Colony color variations from red to pink-white may occur in colonies of *Salmonella* spp. Variations in color are dependent upon the length of incubation and the strain of the organism.

Other non-lactose-fermenting or slow-lactose-fermenting organisms may grow on the agar and imitate the enteric pathogens.[8]

Brilliant Green Agar is not recommended for isolation of *Salmonella* Typhi, *Salmonella* Paratyphi, and *Shigella* spp.

The color of the medium may shift during shipment. This is normal and should not affect the performance of the medium. Placing plates in refrigerated conditions (2-8°C) upon receipt overnight will result in the medium returning to its normal appearance. The medium may become discolored if exposed to light.

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	
Salmonella enterica ATCC® 14028	A	18-24hr	35°C	Aerobic	Growth; red to pink-white colonies with red zone
Escherichia coli ATCC® 25922	B	18-24hr	35°C	Aerobic	Partial to complete inhibition; small yellow to yellow-green colonies

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

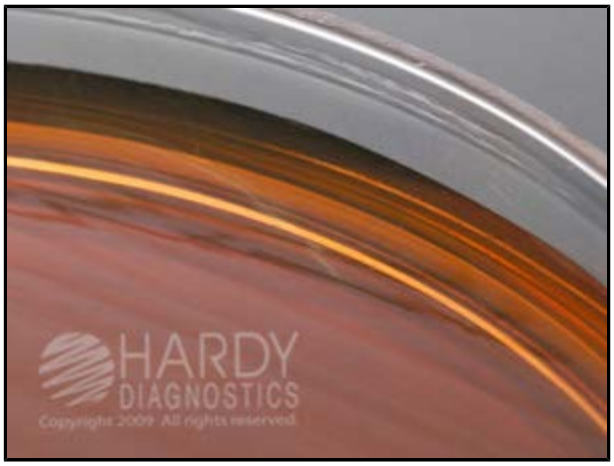
USER QUALITY CONTROL

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

Brilliant Green Agar should appear slightly opalescent, and orange- to green-brown in color.

Salmonella enterica (ATCC® 14028) colonies growing on Brilliant Green Agar (Cat. no. G75). Incubated aerobically for 24 hours at 35ºC.



Escherichia coli (ATCC® 25922) growth inhibited on Brilliant Green Agar (Cat. no. G75). Incubated aerobically for 24 hours at 35ºC.

REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

2. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.

3. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.

4. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I & II. American Society for Microbiology, Washington, D.C.

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

6. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

7. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.

8. Sack, R.B., et al. 1980. *Cumitech 12;* American Society for Microbiology, Washington, D.C.

9. Kristensen, M., et al. 1925. *Br. J. Exp. Pathol.* ; 6:291.

10. Kauffmann, F. 1935. *Z. Hyg. Infektionskr.*; 117:26.

IFU-10083[B]

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]