

Instructions for Use

BRILLIANT GREEN BILE BROTH WITH DURHAM TUBE

Cat. no. K09	Brilliant Green Bile Broth with Durham Tube, 20x125mm Tube with Screw Cap, 13ml	20 tubes/box
Cat. no. K66	Brilliant Green Bile Broth with Durham Tube, 16x125mm Tube with Screw Cap, 10ml	20 tubes/box

INTENDED USE

Hardy Diagnostics Brilliant Green Bile Broth with Durham Tube is recommended for the detection of coliforms in water and other samples.

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

SUMMARY

The formulation for Brilliant Green Bile Broth was developed by the Association of Official Analytical Communities (AOAC) and the American Public Health Association (APHA).⁽³⁻⁷⁾ The basal medium is composed of peptone, which contains 2% bile, and brilliant green dye. Bile is inhibitory to gram-positive microorganisms, while brilliant green dye inhibits selected gram-negative bacilli. Lactose-fermenting organisms resistant to these inhibitors are detected by the production of gas. Gas production is noted by the appearance of bubbles in the durham tube.

FORMULA

Ingredients per liter of deionized water:*

Oxbile	20.0gm
Pancreatic Digest of Gelatin	10.0gm
Lactose	10.0gm
Brilliant Green	13.3mg

Final pH 7.2 +/- 0.2 at 25°C.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt, store media at 2-30°C. 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

Specimen Collection: Consult listed references for information on specimen collection.⁽³⁻⁶⁾ 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 and refrigerated until inoculation.

Method of Use: Allow medium to warm to room temperature prior to inoculation. Consult listed references for information concerning inoculation procedures.⁽⁴⁻⁶⁾

INTERPRETATION OF RESULTS

Production of gas within 48 +/- 2 hours of incubation is considered positive evidence of fermentation by coliform bacilli. Absence of gas production within the durham tube in 48 hours is considered a negative test for coliforms. Consult listed references for detailed results for the enumeration of coliforms using Brilliant Green Bile Broth.⁽³⁻⁵⁾

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.

It may be necessary to invert the tube prior to inoculation if bubbles are trapped in the durham tube. Trapped bubbles that are not released may lead to false-positive results.

Turbidity alone is not indicative of a positive test for the presence of coliforms; turbidity with gas production is considered a positive test.

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	
<i>Escherichia coli</i> ATCC® 25922	A	18-48hr	35°C	Aerobic	Growth; gas bubble in durham tube
<i>Salmonella enterica</i> ATCC® 14028	A	18-48hr	35°C	Aerobic	Growth; no gas bubble in durham tube
<i>Enterococcus faecalis</i> ATCC® 29212	B	18-48hr	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

Brilliant Green Bile Broth should appear clear and light green in color.



Escherichia coli (ATCC® 25922) growing in Brilliant Green Bile Broth (Cat. no. K09). The bubble in the Durham tube indicates gas production. Incubated aerobically for 24 hours at 35°C.



Salmonella enterica (ATCC® 14028) growing in Brilliant Green Bile Broth (Cat. no. K09). Incubated aerobically for 24 hours at 35°C.



Enterococcus faecalis (ATCC® 29212) inhibited in Brilliant Green Bile Broth (Cat. no. K09). Incubated aerobically for 24 hours at 35°C.

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

D.C.

5. American Public Health Association. *Standard Methods for the Examination of Water and Wastewater*, APHA, Washington, D.C.
6. "Public Health Service Drinking Water Standards". 1962. Publication No. 956, U.S. Government Printing Office, Washington, D.C.
7. Association of Official Analytical Communities. *Official Methods of Analysis*. AOAC, Washington, D.C.

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

IFU-10084[A]



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