

BRILLIANT GREEN BILE BROTH 2% (7119)

Intended Use

Brilliant Green Bile Broth 2% is used for the detection of coliform bacteria in water, food, and dairy products.

Product Summary and Explanation

The coliform group of bacteria includes aerobic and facultative anaerobic, Gram-negative, non-sporeforming bacilli that ferment lactose and form acid and gas at 35°C within 48 hours. Members of the *Enterobacteriaceae* comprise the majority of this group, but organisms such as *Aeromonas* spp. may also be included. Procedures to detect and confirm coliforms are used in testing water, foods, dairy products and other materials.¹⁻⁵ Brilliant Green Bile Broth 2% is used to confirm a positive presumptive test result.

Brilliant Green Bile Broth 2% is also referred to as Brilliant Green Bile Broth, Brilliant Green Lactose Broth, Brilliant Green Lactose Bile Broth and Brilliant Green Lactose Bile Broth, 2%.

Principles of the Procedure

Enzymatic Digest of Gelatin is the carbon and nitrogen source used for general growth requirements in Brilliant Green Bile Broth 2%. Oxbile and Brilliant Green inhibit Gram-positive bacteria and many Gram-negative bacteria, other than coliforms. Lactose is a carbohydrate source. Bacteria that ferment lactose and produce gas are detected.

Formula / Liter

Enzymatic Digest of Gelatin	10 g
Lactose	10 g
Oxbile	20 g
Brilliant Green	0.0133 g

Final pH: 7.2 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 40 g of the medium in one liter of purified water until evenly dispersed.
2. Heat with frequent agitation to completely dissolve the medium.
3. Distribute into fermentation tubes.
4. Autoclave at 121°C for no longer than 15 minutes. To avoid entrapment of bubbles in the fermentation tubes, allow the autoclave to cool at least to 75°C before opening.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and green-beige.

Prepared Appearance (1X): Prepared medium is emerald green and clear with none to light precipitate.

Prepared Appearance (2X): Prepared medium is very dark green with brown highlights and clear, with none to light precipitate.

Expected Cultural Response: Cultures listed below are inoculated into Brilliant Green Bile Broth 2% and incubated at appropriate atmosphere and temperatures and examined for growth at 18 – 48 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Result	
		Growth	Gas
<i>Klebsiella pneumoniae</i> ATCC® 13883	10 - 300	Good to excellent	Positive
<i>Enterococcus faecalis</i> ATCC® 29212	~ 1000	Marked to complete inhibition	Negative
<i>Escherichia coli</i> ATCC® 25922	10 - 300	Good to excellent	Positive
<i>Staphylococcus aureus</i> ATCC® 25923	~ 1000	Marked to complete inhibition	Negative

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific instructions for the material being tested. ¹⁻⁵

1. Subculture from a presumptive positive coliform specimen in Lauryl Sulfate Broth or from typical coliform colonies on Violet Red Bile Agar to tubes of Brilliant Green Bile Broth 2%.
2. Incubate at 35°C for 48 ± 2 hours.
3. Examine for bubbles (gas) in the fermentation tube.

Results

Positive: Bubbles (gas) in fermentation tube.

Negative: No bubbles (gas) in fermentation tube.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Brilliant Green Bile Broth 2% Code No.	7119A	500 g
	7119B	2 kg
	7119C	10 kg

References

1. **U. S. Food and Drug Administration.** Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
2. **Cunnif, P. (ed.).** 1995. Official Methods of Analysis AOAC International, 16th ed. AOAC International, Gaithersburg, MD.
3. **Vanderzant, C., and D. F. Splittstoesser (eds.).** Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
4. **Marshall, R. T. (ed.).** Standard methods for the examination of dairy products, 16th ed., American Public Health Association, Washington, D.C.
5. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.