



# PRODUCT SPECIFICATION SHEET

## Brilliant Green Bile Broth-2% (DM046)

### Intended Use

Brilliant Green Bile Broth-2% / Brilliant Green Lactose Bile Broth (DM046) is used for the detection of coliform organisms in foods, dairy products, water and wastewater, as well as in other materials of sanitary importance.

### Product Summary and Explanation

Brilliant Green Bile Broth 2% is formulated according to the American Public Health Association (APHA)<sup>(4)</sup> specifications for use in the confirmation of presumptive tests for coliforms. 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.<sup>(1-5)</sup> 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

Ingredients	Gms / Litre
Enzymatic Digest of Gelatin	10.00
Lactose	10.00
Oxbile	20.00
Brilliant Green	0.0133
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 only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

### Directions

1. Suspend 40 g of the medium in one liter of distilled water.
2. Heat to completely dissolve the medium.
3. Distribute into tubes containing inverted fermentation Durham vials.
4. Autoclave at 121°C , 15 psi pressure, for 15 minutes.
5. To avoid entrapment of bubbles in the fermentation tubes, allow the autoclave to cool at least to 75°C before opening.





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### Quality Control Specifications

<b>Dehydrated Appearance</b>	Light green beige, homogeneous, free flowing powder
<b>Solution</b>	4% Solution in Distilled or deionized water is soluble on boiling, emerald green colored, and clear.
<b>Prepared Medium</b>	Emerald green colored clear solution.
<b>Reaction of 4.0% Solution</b>	pH 7.2 $\pm$ 0.2 at 25°C
<b>Gel Strength</b>	Not Applicable

**Expected Cultural Response:** Cultural response in Brilliant Green Bile Broth-2% after incubation at 35-37°C for 18 - 48 hours.

Sr. No.	Organisms	Results to be achieved		
		Inoculum (CFU)	Growth	Gas
1.	<i>Bacillus cereus</i> ATCC 10876	$\geq 10^3$	inhibited	--
2.	<i>Escherichia coli</i> ATCC 25922	50 -100	good-luxuriant	positive reaction
3.	<i>Enterobacter aerogenes</i> ATCC 13048	50 -100	good-luxuriant	positive reaction
4.	<i>Enterococcus faecalis</i> ATCC 29212	50 -100	none-poor	negative reaction
5.	<i>Staphylococcus aureus</i> ATCC 25923	$\geq 10^3$	inhibited	--

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.<sup>(1-5)</sup>

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  $\pm$  2 hours.
3. Examine for bubbles (gas) in the fermentation tube.

### Results

Examine the medium for growth and gas production after 18 - 24 and 48 hours incubation.

Positive: Bubbles (gas) in fermentation tube.

Negative: No bubbles (gas) in fermentation tube.





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Uninoculated  
Tube



*Escherichia coli*  
ATCC 25922

### Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

### Expiration

Refer to the 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.

### Limitations of the Procedure

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

### Packaging

**Product Name: Brilliant Green Bile Broth-2%**

**Product Code : DM046**

**Available Pack sizes : 100gm / 500gm**

### References

1. U. S. Food and Drug Administration. 1995. 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.). 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
4. Marshall, R. T. (ed.). 1993. Standard methods for the examination of dairy products, 16th ed., American Public Health Association, Washington, D.C.





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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.

### Further Information

For further information please contact your local MICROMASTER Representative.



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