

## BRILLIANT GREEN BILE BROTH 2% W/ MUG (7344)

### **Intended Use**

**Brilliant Green Bile Broth 2% W/ MUG** is used for the detection of coliforms and the fluorogenic detection of *Escherichia coli*.

### **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. *Escherichia coli* is a member of the fecal coliform group. The presence of *E. coli* is indicative of fecal contamination.<sup>1</sup> Procedures to detect and confirm the presence of coliforms are used in testing water, foods, dairy products, and other materials.<sup>1-5</sup> The procedures begin with a presumptive test, and when positive, confirmed using Brilliant Green Bile Broth 2%. The addition of the fluorogenic compound, MUG (4-Methylumbelliferyl-®-D-glucuronide) to this medium permits the rapid detection of *E. coli* when medium is observed for fluorescence using a long-wave UV light source.

### **Principles of the Procedure**

Enzymatic Digest of Gelatin provide the nitrogen and vitamin sources in Brilliant Green Bile Broth 2% W/ MUG. Lactose is the carbohydrate energy source. Oxbile and Brilliant Green inhibit Gram-positive bacteria and many Gram-negative bacteria, other than coliforms. The substrate MUG (4-Methylumbelliferyl-®-D-glucuronide) produces a blue fluorescence when hydrolyzed by the enzyme ®-glucuronidase, produced by most *E. coli*.

### **Formula / Liter**

Enzymatic Digest of Gelatin ..... 10 g  
 Lactose ..... 10 g  
 Oxbile ..... 20 g  
 MUG (4-Methylumbelliferyl-®-D-glucuronide) ..... 0.05 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.
2. Dispense into tubes containing inverted fermentation tubes.
3. Autoclave at 121°C for 15 minutes.

### **Quality Control Specifications**

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

**Prepared Appearance:** Prepared medium is emerald green and clear.

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

Microorganism	Response	Reactions	
		Gas	Fluorescence
<i>Enterobacter aerogenes</i> ATCC® 13048	growth	positive	negative
<i>Enterococcus faecalis</i> ATCC® 29212	inhibited	negative	negative
<i>Escherichia coli</i> ATCC® 25922	growth	positive	positive
<i>Staphylococcus aureus</i> ATCC® 25923	inhibited	negative	negative

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

### Test Procedure

Follow the methods and procedures for the test being performed using Brilliant Green Bile Broth 2% W/ MUG.

### Results

Observe inoculated tube for characteristic growth, gas production, and fluorescence following incubation. Positive MUG reactions exhibit a bluish fluorescence throughout the tube when exposed to long wave UV light. Non-*E.coli* coliforms may produce gas but do not fluoresce.

### Storage

Store 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 appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

### Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Glucuronidase-negative strains of *E. coli* have been encountered<sup>6-9</sup> and will be missed by this procedure.
3. Strains of *Salmonella* spp. and *Shigella* spp. that produce glucuronidase may infrequently be encountered.<sup>10</sup> These strains must be distinguished from *E. coli* on the basis of other parameters, i.e., gas production, lactose fermentation, or growth at 44.5°C.

### Packaging

Brilliant Green Bile Broth 2% W/ MUG	Code No.	7344A	500 g
		7344B	2 kg
		7344C	10 kg

### References

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4. **Hitchins, A. D., P. Feng, W. D. Watkins, S. R. Rippey, and L. A. Chandler.** 1995. *Escherichia coli* and the coliform bacteria, p. 4.01-4.29. *In* Bacteriological analytical manual, 8<sup>th</sup> ed. AOAC International, Gaithersburg, MD.
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6. **Chang, G. W., J. Brill, and R. Lum.** 1989. Proportion of  $\beta$ -D-Glucuronidase-negative *Escherichia coli* in human fecal samples. *Appl. Environ. Microbiol.* **55**:335-339.
7. **Hansen, W., and E. Yourassowsky.** 1984. Detection of  $\beta$ -D-Glucuronidase in lactose fermenting members of the family enterobacteriaceae and its presence in bacterial urine cultures. *J. Clin. Microbiol.* **20**:1177-1179.
8. **Kilian, M. and P. Bulow.** 1976. Rapid diagnosis of *Enterobacteriaceae*. *Acta. Pathol. Microbiol. Scand. Sect. B* **84**:245-251.
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10. **Damare, J. M., D. F. Campbell, and R. W. Johnston.** 1985. Simplified direct plating method for enhanced recovery of *Escherichia coli* in food. *J. Food Sc.* **50**:1736-1746.

### 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.