

## VIOLET RED BILE AGAR W/ MUG (7359)

### Intended Use

**Violet Red Bile Agar W/ MUG** is used for the detection of coliforms and the fluorogenic detection of *Escherichia coli*.

### Product Summary and Explanation

Violet Red Bile Agar W/ MUG is specified in many procedures to enumerate coliforms in food and dairy products.<sup>1-3</sup> In 1982, Feng and Hartman developed a rapid fluorogenic assay for *Escherichia coli* by incorporating 4-methylumbelliferyl- $\beta$ -D-glucuronide (MUG) into Lauryl Tryptose Broth.<sup>4</sup> Incorporating MUG into Violet Red Bile Agar permits the detection of *E. coli* among the coliform colonies.<sup>2,3</sup> Standard methods procedures specify Violet Red Bile Agar with MUG for detecting *E. coli* in food and dairy products by fluorescence.<sup>1-3</sup> This medium is often abbreviated as VRBA w/ MUG.

### Principles of the Procedure

Enzymatic Digest of Gelatin is the source of carbon, nitrogen, vitamins, and minerals in Violet Red Bile Agar w/ MUG. Yeast Extract supplies B-complex vitamins that stimulate bacterial growth. Bile Salts Mixture and Crystal Violet inhibit Gram-positive bacteria. Lactose is a carbohydrate source. Neutral Red is a pH indicator. MUG (4-methylumbelliferyl- $\beta$ -D-glucuronide) is a substrate used for detecting glucuronidase activity. Agar is the solidifying agent.

### Formula / Liter

Enzymatic Digest of Gelatin .....	7 g
Yeast Extract.....	3 g
Bile Salts Mixture .....	1.5 g
Lactose .....	10 g
Sodium Chloride .....	5 g
Neutral Red.....	0.03 g
Crystal Violet.....	0.002 g
4-Methylumbelliferyl- $\beta$ -D-Glucuronide .....	0.1 g
Agar .....	15 g

Final pH: 7.4  $\pm$  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. Suspend 41.6 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for only two minutes to completely dissolve the medium.
3. Cool the medium to 45 - 46°C and pour plates.

### Quality Control Specifications

**Dehydrated Appearance:** Powder is homogeneous, free flowing, and pink to red-beige.

**Prepared Appearance:** Prepared medium is trace to slightly hazy and red-purple.

**Expected Cultural Response:** Cultural response on Violet Red Bile Agar w/ MUG incubated aerobically at 35 ± 2°C and examined for growth after 18 - 24 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Growth	Fluorescence
<i>Enterobacter aerogenes</i> ATCC® 13048	10 - 300	Good growth	Negative
<i>Escherichia coli</i> ATCC® 25922	10 - 300	Good growth	Positive
<i>Salmonella typhimurium</i> ATCC® 14028	10 - 300	Good growth	Negative
<i>Staphylococcus aureus</i> ATCC® 25923	1000	Inhibited	Negative

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

### **Test Procedure**

1. Process each specimen as appropriate.<sup>1-3</sup>
2. Incubate plates at 35°C for 22 - 26 hours.
3. Examine plates for growth and fluorescence.

### **Results**

Coliform organisms form purple-red colonies that are generally surrounded by a reddish zone of precipitated bile. When examined under long-wave fluorescent light, MUG-positive colonies are surrounded by a bluish fluorescent halo. MUG-negative colonies lack the fluorescent halo. *E. coli* colonies are red surrounded by a zone of precipitated bile and fluoresce blue under long-wave UV light. *Salmonella* and *Shigella* strains that produce glucuronidase may be encountered infrequently, but these are generally lactose negative and appear as colorless colonies which may fluoresce.

### **Storage**

Store sealed bottle containing the dehydrated medium at 2 - 30°. 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.

### **Limitations of the Procedure**

1. Due to varying nutritional requirements, 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>5-7</sup> Similarly, glucuronidase-positive strains of *E. coli* that do not fluoresce have been reported.<sup>8</sup> Strains of *Salmonella* and *Shigella* that produce glucuronidase may infrequently be encountered.<sup>9</sup> These strains must be distinguished from *E. coli* on the basis of other parameters, e. g., gas production, lactose fermentation or growth at 44.5°C.

### **Packaging**

Violet Red Bile Agar w/ MUG	Code No.	7359A	500 g
		7359B	2 kg
		7359C	10 kg

### **References**

1. **Marshall, R. T. (ed.).** 1993. Standard methods for the examination of dairy products, 16<sup>th</sup> ed. American Public Health Association, Washington, D.C.
2. **Vanderzant, C. and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3<sup>rd</sup> ed. American Public Health Association, Washington, D.C.
3. [www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm](http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm).

4. **Feng, P. C. S., and P. A. Hartman.** 1982. Fluorogenic assays for immediate confirmation of *Escherichia coli*. Appl Environ. Microbiol. **43**:1320-1329.
5. **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.
6. **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.
7. **Kilian, M., and P. Bulow.** 1976. Rapid diagnosis of *Enterobacteriaceae*. Acta. Pathol. Microbiol. Scand. Sect. B, **84**:245-251.
8. **Mates, A., and M. Shaffer.** 1989. Membrane filtration differentiation of *E. coli* from coliforms in the examination of water. J. Appl. Bacteriol. **67**:343-346.
9. **Damare, J. M., D. F. Campbell, and R. W. Johnston.** 1985. Simplified direct plating method for enhanced recovery of *Escherichia coli* in food. J. of Food Sci. **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.