

## 16016 BRILA MUG Broth (Brilliant Green 2%-Bile MUG Broth, Brilliant Green Bile Lactose MUG Broth)

Fluorescent method for the detection of *E. coli* and coliforms. Bile and brilliant green extensively inhibit the growth of accompanying flora, in particular gram-positive microorganisms. The presence of *E. coli* results in fluorescence in the UV. A positive indole test and possibly gas formation from lactose fermentation provide confirmation. The broth can be used in conjunction with the MPN method for *E. coli* and coliform enumeration in the water of bathing areas.

### Composition:

Ingredients	Grams/Litre
Peptone	10.0
Lactose	10.0
Ox-bile	20.0
Brilliant green	0.133
L-Tryptophan	1.0
4-Methylumbelliferyl- $\beta$ -D-glucuronide	0.1
Final pH 7.2 +/- 0.2 at 37°C	

Store prepared media below 8°C, protected from direct light. Store dehydrated powder, in a dry place, in tightly-sealed containers at 2-25°C.

### Directions:

Dissolve 41 g in 1 litre distilled water and pour into tubes fitted with Durham tubes if necessary. Autoclave at 121°C.

Gas production from lactose fermentation is indicated by using inverted Durham tubes. Inoculate at least 1 ml sample in a tube containing 10 ml of BRILA MUG Broth and a Durham tube. Incubate at 37°C for 16-24 hours. In case of gas formation the Durham tubes rise or/and show bubbles. If no gas has formed in the inverted tube, reincubate and reexamine after 48 hours. Turbidity of the medium accompanied by formation of gas within 48 hours is a positive presumptive test for the presence of *E. coli* and/or other coliform organisms. For further confirmation incubate at 44°C, where *E. coli* still can grow.

In addition check the tubes under UV light at 360-370 nm. Light blue fluorescence indicates the presence of *E. coli*. If there is no fluorescence after 24 hours of incubation do NOT add Kovacs reagent (Fluka 60983) to check the indole reaction because this alcoholic reagent destroys the growth conditions for cultures. Continue incubation for another 24 hours and check again for fluorescence and indole reaction. For confirmation add Kovacs reagent to the tubes (5 mm layer). If the reagent layer becomes cherry red after 1-2 minutes, the presence of *E. coli* is confirmed.

### Principle and Interpretation:

Peptone act as a source of nitrogen, carbon, vitamins and amino acids. Lactose is the fermentable sugar. Ox-bile and Brilliant green suppress the accompanying microbial flora in particular gram-positive organisms. Lactose-positive bacteria metabolize lactose with gas formation, within 24 hour or less is a presumptive evidence of the presence of coliform bacteria. The addition of tryptophan improves the indole reaction.  $\beta$ -D-glucuronidase, which is produced by *E. coli*, cleaves 4-Methylumbelliferyl- $\beta$ -D-glucuronide to 4-methylumbelliferone and glucuronide. The fluorogen 4-methylumbelliferone can be detected under a long wavelength UV lamp.

Cultural characteristics after 24-48 hours at 35°C .

Organisms (ATCC)	Growth	Fluorescence	Gas formation	Indole reaction
<i>Escherichia coli</i> (25922)	+++	+	+	+
<i>Citrobacter freundii</i> (11775)	+++	-	-	-
<i>Bacillus cereus</i> (11778)	-			
<i>Lactobacillus plantarium</i> (8014)	-			
<i>Micrococcus luteus</i> (10240)	-			
<i>Staphylococcus aureus</i> (25923)	-			

References:

1. G. Havemeister, et al., Screening of bathing-water for coliforms according to EC directive, Zbl. Hyg. 191, 523 (1991)