BRILLIANT GREEN BILE BROTH 2% ISO 4831, ISO 4832

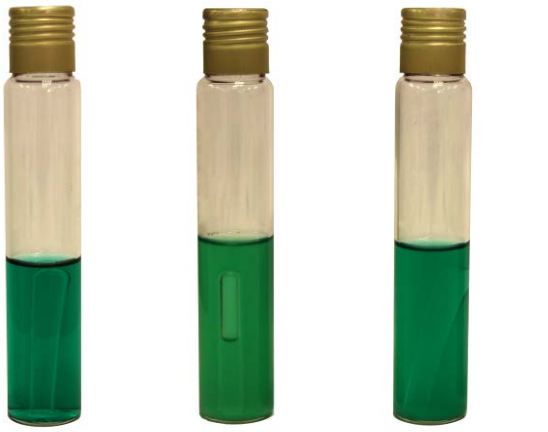
CAT Nº: 1228

For the detection of coliforms in water and foods

FORMULA IN g/l

Ox bile	20.00	Lactose	10.00
Gelatin Peptone	10.00	Brilliant Green	0.0133

Final pH 7.2 ± 0.2 at 25°C



Uninoculated Tube | *Escherichia coli* ATCC 25922 | *Enterobacter aerogenes* ATCC 13048

PREPARATION

Suspend 40 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into tubes with Durham gas collecting tubes for gas detection and sterilize in autoclave at 121°C for 15 minutes. **AVOID OVERHEATING**. The prepared medium should be stored at 2-8°C. The color is brilliant green.

- When the sample has 1 ml or less volume, dispense medium in volumes of 10 ml.
- To analyze samples of 10 ml, dissolve 80 grams of the medium in a liter of distilled water. Distribute in the same manner.

The dehydrated medium should be homogeneous, free-flowing and beige-greenish in color. If there are any physical changes, discard the medium.

USES

BRILLIANT GREEN BILE BROTH 2% is a selective medium recommended by APHA for the cultivation of coliforms in drinking water, wastewater, foods and dairy products, and other products of sanitary concern.
It is used as a confirmation test in procedures where presumptive tests for presence of coliforms are positive. The production of gas both at 37°C and 44.5°C confirms the presence of coliforms.

The Gelatin peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Lactose is the fermentable carbohydrate providing carbon and energy. Ox bile and Brilliant green inhibit Gram-positive bacteria and most Gram-negative bacteria except coliforms. They also prevent the growth of the anaerobic lactose-fermenters such as *Clostridium perfringens*, which could give false positive reactions at 44.5°C.

Inoculate the medium and incubate at 37°C and 44.5°C for 24 - 48 hours. For milk analysis incubation at 32°C is recommended. The presence of gas is considered a positive test for the coli-enterobacter group.

This medium is recommended by ISO 4831 and ISO 4832 normatives for the confirmation of coliforms. From an incubated tube of Lauryl Sulfate Broth – Lauryl Tryptose Broth (Cat.1310) inoculate a tube of confirmation medium (Brilliant green bile broth 2 %). Incubate at 30 °C or 37 °C for 24 h ± 2 h or, if gas formation is not carried out, after 48 h ± 2 h). A tube in which gas formation is observed after 24 h ± 2 h or 48 h ± 2 h is considered as a positive tube.

To indicate the presence of *Escherichia coli*, incubate Brilliant Green Bile Broth 2% at 44 ± 1°C for 48 hours. Turbidity in the broth and gas production in the inverted tube are positive signs. An indole production test at 44.5°C is also carried out in Peptone-Tryptone Water (Cat.1403) to confirm *Escherichia coli.*

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 37°C and 44.5°C and observed after 24 - 48 hours.

Microorganisms	Growth	Gas Production 37°C	Gas Production 44.5°C
**Escherichia coli* ATCC 25922	Good	+	+
Enterobacter aerogenes ATCC 13048	Good	+	-
Staphylococcus aureus ATCC 25923	Inhibited	-	-
Enterococcus faecalis ATCC 19433	Inhibited	-	-

*According ISO 4831– ISO 4832 incubate at 30 or 37°C for 24 ± 2 hours and 48 ± 2 hours

According to ISO 11133 24-48h/30±1°C (Productivity, Selectivity)

Microorganisms	Inoculum (cfu/ml)	Productivity Qualitative	Selectivity Qualitative	Characteristic Reaction
Escherichia coli ATCC 8739	$10\text{-}10^2$	Turbidity (2) and gas in Durham tube		Gas production and Turbidity
Escherichia coli ATCC 25922	$10\text{-}10^2$	Turbidity (2) and gas in Durham tube		Gas production and Turbidity
Citrobacter freundii ATCC 43864	$10\text{-}10^2$	Turbidity (2) and gas in Durham tube		Gas production and Turbidity
Enterococcus faecalis ATCC 29212	10^3 -10^4		Inhibited without gas production	

BIBLIOGRAPHY

Standard Methods for the Examination of Water and Sewage, 9th. Edition 195. 1946. Standard Methods for the Examination of Dairy Products, 9th. Edition 152. 1948.

ISO

STORAGE

Once opened keep powdered medium closed to avoid hydration.