

# Technical Data Sheet

## MOLEQULE-ON®

### Brilliant Green Bile Broth 2% ISO

Cat #: MM-M-N228

For the confirmation of coliforms in water and foods

#### Principles and 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 the presence of coliforms are positive.

Enzymatic digest of casein 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.

This medium is recommended by ISO 4831 and ISO 4832 normatives for the confirmation of coliforms. The production of gas at 30°C or 37°C confirms the presence of coliforms.

#### Formula per Litre:

Enzymatic digest of casein	10g	Brilliant green	0.0133g
Lactose	10g	Ox Bile	20g

#### 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. Cool to 50°C, mix well and dispense into plates. AVOID OVERHEATING.

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 and distribute in the same manner.

#### Instructions for use:

For the confirmation of coliforms according to ISO 4831 and 4832:

- Inoculate the typical colonies from the incubated plates of VRBL (Cat. MM-M-N093), into tubes of Brilliant Green Bile Broth 2%.
- Incubate the tubes at a temperature of 30°C or 37°C.
- Consider as coliforms colonies that show gas formation in the Durham tubes.

For the confirmation 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. MM-M-N403).

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### Quality control:

Solubility	Appearance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25°C)
w/o rests	Fine powder	Beige-greenish	Brilliant green	7.2 ± 0.2

### Microbiological test:

According to ISO 11133:

Incubation conditions: (24±2, 48±2 h / 30±1 °C).

Inoculation conditions: Productivity qualitative (<100 CFU) / Selectivity (10<sup>4</sup>-10<sup>6</sup> CFU).

Microorganisms	Specification	Characteristic reaction
Escherichia coli ATCC 25922	Turbidity (2) and gas in Durham tube	Gas production and turbidity
Enterococcus faecalis ATCC 29212	Partial inhibition without gas production	
Citrobacter freundii ATCC 43864	Turbidity (2) and gas in Durham tube	Gas production and turbidity
Escherichia coli ATCC 8739	Turbidity (2) and gas in Durham tube	Gas production and turbidity

### Storage:

Temperature: 2°C - 25°C

### Bibliography:

ISO 4831: 2006 Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of coliforms — Most probable number technique.

ISO 4832:2006 Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coliforms — Colony-count technique. 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.