# BRILLIANT GREEN BILE BROTH 2%

BRGR-00I-500

- **Principle**

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 normative for the confirmation of coliforms. The production of gas at 30 °C or 37 °C confirms the presence of coliforms.

- **Regulatory compliance**

This product is manufactured under a quality management system in accordance with ISO 9001 and ISO 13485, and its formulation and quality control comply with applicable international standards, such as ISO 11133, where relevant.

For this specific medium, compliance is also established with the relevant requirements of ISO 4831 and ISO 4832.

- **Composition**

| **Ingredients** | **g/L** |
|---|---|
| Enzymatic digest of casein | 10.00 |
| Lactose | 10.00 |
| Brilliant green | 0.0133 |
| Ox bile | 20.00 |

- **Preparation**

Suspend 40 grams of the medium in one litre 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.

When the sample has 1 ml or less volume, dispense medium in volumes of 10 ml. To analyse samples of 10 ml, dissolve 80 grams of the medium in a litre of distilled water and distribute in the same manner.

- **Applications and use**

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

- Inoculate the typical colonies from the incubated plates of VRBL (ABVL-00I-500), 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.

- **Quality control**

| | |
|---|---|
| **Solubility** | w/o rests |
| **Appearance** | Fine powder |
| **Colour of the dehydrated medium** | Beige-greenish |
| **Colour of the prepared medium** | Brilliant green |
| **Final pH (25 °C)** | 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 ($10^4$-$10^6$ CFU).

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

- **Storage**

The product is highly hygroscopic; keep the container always closed and store it properly as per the conditions mentioned on the label. The declared expiry is valid only when stored as per the conditions mentioned on the label. Temp. Min.:2 °C Temp. Max.:25 °C.

Note: Sterilize media immediately after reconstitution.

- **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.

- **Product use limitation**

This product is developed, designed and supplied exclusively for research use only. It is not intended for diagnostic applications or drug development, and it is not suitable for administration to humans or animals.