



BRILLIANT GREEN BILE BROTH 2%

Dehydrated and ready-to-use culture medium



Brilliant Green Bile Broth 2% - from the left:
E.coli, *K.pneumoniae*, *Salmonella* sp.

1 - INTENDED USE

Selective medium for the confirmatory test of coliforms in foodstuffs.

2 - COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

| | |
|-----------------|---------|
| Oxgall | 20.0 g |
| Lactose | 10.0 g |
| Peptone | 10.0 g |
| Brilliant green | 13.3 mg |

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Brilliant Green Bile Broth 2% is a modification of MacConkey's liquid medium, formulated by Dunham and Schoenlein¹ in 1926 to attain the maximum recovery of bacteria of the coli-aerogenes group, while inhibiting most Gram-positive organisms.²

Brilliant Green Bile Broth 2% is recommended by ISO 4831, ISO 4832, FDA-BAM for the confirmatory test of coliform bacteria in foods and by APHA for the confirmatory test of coliforms in waters.³⁻⁶

Peptone provides nitrogen, carbon and minerals for microbial growth; lactose is a fermentable carbohydrate. Brilliant green inhibits Gram-positive organisms and some Gram-negative bacteria other than coliforms, while oxgall, which is a mixture of bile salts, provides an added suppressive effect on Gram-positive organisms; brilliant green and oxgall also suppress the growth of the anaerobic lactose-fermenting bacteria, which could give false positive reactions. Coliforms, which are resistant to the inhibitory effects of both brilliant green and oxgall, at 30 °C or 37 °C cause fermentation of lactose with the production of gas.

4 - DIRECTIONS FOR MEDIUM PREPARATION

Suspend 40 g in 1000 mL of cold purified water; heat slightly to completely dissolve the powder, mix well and distribute 10 mL into test tubes containing inverted Durham tube. Sterilise by autoclaving at 121°C for 15 minutes. Cool the broth as quickly as possible. With inocula greater than 1 mL for 10 mL of medium, use multiple strength medium and sterilise at 100°C for 30 minutes.

5 - PHYSICAL CHARACTERISTICS

| | |
|------------------------------|--|
| Dehydrated medium appearance | grey-green, fine, homogeneous, free-flowing powder |
| Prepared tubes appearance | blue-green, limpid |
| Final pH at 20-25 °C | 7.2 ± 0.2 |

6 - MATERIALS PROVIDED - PACKAGING

| Product | Type | REF | Pack |
|-------------------------------|--------------------|---------|------------------------------|
| Brilliant Green Bile Broth 2% | Dehydrated medium | 4012652 | 500 g (12.5 L) |
| | | 4012654 | 5 kg (125 L) |
| Brilliant Green Bile Broth 2% | Ready-to-use tubes | 551265 | 20 x 10 mL with Durham tubes |

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops, incubator and laboratory equipment as required, Erlenmeyer flasks, test-tubes, Durham tubes, ancillary culture media and reagents.

8 - SPECIMENS

Food and water samples. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

9 - TEST PROCEDURE

For the confirmation of coliform bacteria enumerated with the most probable number (MPN) technique³ proceed as following:

From each of the incubated tubes with single strength and double-strength Lauryl Pepto Bios Broth (REF 401580) inoculate with a loop a tube of Brilliant Green Bile Broth and incubate at 30°C or 37°C for 24 h ± 2 h or, if gas formation is not observed, for 48 ± 2 h.

For the confirmation of coliform bacteria enumerated with the colony-count technique⁴ proceed as following:

Inoculate five colonies of each doubtful type cultivated on Violet Red Bile Lactose Agar (REF 402185), into tubes of Brilliant Green Bile Broth 2%. Incubate the tubes at 30°C or 37°C for 24 h ± 2 h.

10 - READING AND INTERPRETATION

The medium becomes turbid and yellowish-green in colour when bacteria are growing; gas formation can be observed as bubbles production accumulated into Durham tubes.

Consider as coliforms the colonies or the growth in liquid media that show gas formation in the Durham tube of Brilliant Green Bile Broth test tubes incubated at 30 or 37°C for 24-48 hours.





11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

| CONTROL STRAINS | INCUBATION T° / T / ATM | EXPECTED RESULTS |
|------------------------------|-------------------------|--|
| <i>E.coli</i> ATCC 25922 | 30°C/24 H/A | growth, with gas production |
| <i>E.coli</i> ATCC 8739 | 30°C/24 H/A | growth, with gas production |
| <i>C.freundii</i> ATCC 43864 | 30°C/24 H/A | growth, with gas production |
| <i>E.faecalis</i> ATCC 19433 | 30°C/24 H/A | growth partially inhibited without gas |

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

12 – PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of dehydrated Brilliant Green Bile Broth 2% is tested for productivity, specificity and selectivity by comparing the results with a previously approved Reference Batch with incubation at 30°C for 24-48 hours.

Productivity is tested by dilution to extinction method, by inoculating 1 mL of appropriate decimal dilutions of target organisms in test tubes, incubating at 30°C for 18-24 hours and recording the highest dilution showing growth and gas production in Reference Batch (Gr_{RB}) and in Test Batch (Gr_{TB}).

Productivity is tested with the following target strains: *E.coli* ATCC 25925, *E.coli* ATCC 8739, *C.freundii* ATCC 43864, *E.aerogenes* ATCC 13048, *K.pneumoniae* ATCC 27736. The productivity index Gr_{RB}-Gr_{TB} for each test strain is ≤ 1 and the tubes exhibit gas into the Durham tubes.

Specificity is tested with appropriate dilutions of non-target strain *S.Typhimurium* ATCC 14028. After incubation the strain exhibits good growth without gas production.

Selectivity is tested with mixtures appropriate dilutions of non-target strains *S.aureus* ATCC 25923 and *E.faecalis* ATCC 19433. After incubation of inoculated tubes the growth of *S.aureus* is totally inhibited while the growth of *E.faecalis* is partially inhibited.

13 - PRECAUTIONS AND WARNINGS

- This product is for microbiological control only and for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- This culture medium contains raw materials of animal origin. The ante and post mortem controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that the product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- Apply Good Manufacturing Practice in the production process of prepared media.
- Be careful when opening screw cap tubes to prevent injury due to breakage of glass.
- Each tube is for single use only; do not transfer or subdivide the tube content in other containers.
- Brilliant Green Bile Broth 2% ready-to-use tubes are sterilized by autoclaving.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized tubes inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheets of the products are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

14 - STORAGE CONDITIONS AND SHELF LIFE

Dehydrated medium

Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).

The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type and the applied storage conditions (temperature and packaging). According to Baird RM et al. the self-prepared tubes can be stored at 4 ± 2°C in screw capped containers for up to four weeks.²

Ready to use tubes

Upon receipt, store tubes in their original pack at +2°C/ +8°C away from direct light. If properly stored, the tubes may be used up to the expiration date. Do not use the tubes beyond this date. Tubes from opened secondary packages can be used up to the expiration date. Opened tubes must be used immediately. Before use, check the closing and the integrity of the screw cap. Do not use tubes with signs of deterioration (e.g., microbial contamination, atypical colour).

15 - REFERENCES














1. Dunham HG, Schoenlein HW. Brilliant Green bile media. *Stain Technol.* 1926; 1:129-134
2. Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. *Int J Food Microbiol* 1987; 206-207.
3. 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
- FDA-BAM Chapter 4: Enumeration of Escherichia coli and the Coliform Bacteria. Content current as of:10/09/2020.
- APHA Standard Methods for the Examination of Water and Wastewater, 23rd ed. 2017.

TABLE OF APPLICABLE SYMBOLS

| | | | | | |
|--|--|--|--|---|---|
|  or  Catalogue number |  Batch code |  Manufacturer |  This side up |  Store in a dry place |  Fragile |
|  Temperature imitation |  Content sufficient for <n> tests |  Consult Instructions for Use |  Use by |  Keep away from direct light |  For single use only |

REVISION HISTORY

| Version | Description of changes | Date |
|------------|----------------------------|---------|
| Revision 2 | Updated layout and content | 2022/06 |

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

