BROMOCRESOL PURPLE GLUCOSE AGAR
(DEXTROSE TRYPTONE AGAR)
Dehydrated culture medium

1 - INTENDED USE
For the enumeration of spores of mesophilic and thermophilic *Bacillus.*

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

Tryptone	10.00 g
Glucose	5.00 g
Soluble starch	2.00 g
Bromocresol purple	0.04 g
Agar	15.00 g

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

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Dextrose Tryptone Agar has been originally devised by Williamas[1] during the studies on the cultivation and enumeration of thermophilic bacteria. In the 1930's, the National Canners Association specified the use of Dextrose Tryptone Agar for isolating "flat sour" organisms from food products. Canned food spoilage, referred to as 'flat-sour' spoilage, is caused by the outgrowth of facultative anaerobic *Geobacillus stearothermophilus, Bacillus coagulans, Bacillus thermoacidurans*. The microbial spoilage of canned food is caused by three reasons: 1) Survival of spores of thermophilic bacteria; 2) Growth of survived thermophilic bacteria due to inadequate cooling, inadequate heat treatment, and improper storage temperature; 3) Recontamination of microorganisms due to can leakage.
In flat-sour spoilage, the foods become sour due to the production of acid from carbohydrates with no can swelling.
Bromocresol Purple Glucose Agar (Dextrose Tryptone Agar) can be used to isolate *Bacillus coagulans* and other mesophilic or thermophilic microbes responsible for food spoilage.
Tryptone provides nitrogen and minerals for microbial growth; glucose is a fermentable carbohydrate and a source of carbon and energy for microbial growth; soluble starch is a protective agent and promotes spore germination; bromocresol purple serves as an acid-base indicator giving a yellow colour to glucose fermenting bacteria while glucose non-fermenting bacteria develop blue colonies.

4 - DIRECTIONS FOR MEDIUM PREPARATION
Suspend 32 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C mix well and distribute into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	purple-grey, fine, homogeneous, free-flowing powder
Prepared plates appearance	purple, limpid
Final pH at 20-25 °C	7.0 ± 0.2

6 - MATERIALS PROVIDED

Product	Type	REF	Pack
Bromocresol Purple Glucose Agar (Dextrose Tryptone Agar)	Dehydrated medium	4012732	500 g (15.6 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED
Autoclave, water-bath, sterile loops, swabs and pipettes, incubator and laboratory equipment as required, Erlenmeyer flasks, sterile Petri dishes, ancillary culture media and reagents.

8 – SPECIMENS
Refer to applicable international standards and regulations for the collection of food samples. Operate in accordance with good laboratory practice for sample collection, storage and transport to the laboratory.

9 - TEST PROCEDURE
The instructions below are included only as guideline of the use of the medium and will vary depending on the origin of the sample and the exact purpose of the test. For more precise details, consult the cited references.[2-6]
Destroy the vegetative cells by heating the sample.
- Inoculate the plates with 1 ml of the sample or of its tenfold dilutions and pour 15 ml of medium into Petri dishes
- Cover and mix the inoculum with the medium.
- Incubate at 30°C for 5 days to enumerate *Bacillus* spores.
- Incubate at 55°C for 5 days to enumerate thermophilic *Bacillus* spores. Pour several drops of sterile paraffin oil in the lid of the plate as a tight seal.

10 - READING AND INTERPRETATION
After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Count the total number of colonies, the total number of acid-producing (yellow halo) colonies and the total number of non-acid producing colonies (blue halo).

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.

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
B.stearothermophilus ATCC 10149	55°C /72H/A	good growth, yellow colonies
B.subtilis ATCC 6633	35-37°C/18-24 H/A	good growth, yellow colonies

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 Bromocresol Purple Glucose Agar is tested for productivity by comparing the results with a previously approved Reference Batch. Productivity is tested by a quantitative test with the target strains *B.stearothermophilus* ATCC 10149, *B.subtilis* ATCC 6633, *B. cereus* ATCC 11778. The plates are inoculated by poured plate method with decimal dilutions in saline of a colonies' suspension and incubated at 55°C (*B.stearothermophilus*) or 35-37°C for 72 (*B.stearothermophilus*) or 18-24 hours. The colonies are enumerated on both batches and the productivity ratio (Pr) is calculated. If Pr is ≥ 0.7 and if the colonies morphology and colour are typical (yellow colonies) the results are considered acceptable and conform to the specifications.

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.
- 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 plates 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 Sheet of the product 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

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.
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 (plates/flasks) and the applied storage conditions (temperature and packaging).

15 - REFERENCES

1. Williams OB.Tryptone medium for the detection of flat-sour spores. Food Res 1936; 1: 217-221.
2. National Canners Association (1993) Bacterial Standards for Sugar.
3. National Canners Association (1968) Laboratory Manual for Food Canners and Processors. Vol.1. p13.
4. National Canners Association (1954) A Laboratory Manual for the Canning Industry' 1st ed., National Canners Association, Washington.
5. Salfinger Y, Tortorello ML (2015) Compendium of Methods for the Microbiological Examination of Foods, 5th EdAmerican Public Health Association, Washington, D.C.
6. Wehr HM, Frank JH (2004), Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

TABLE OF APPLICABLE SYMBOLS

REF Or REF Catalogue number	LOT Batch code	Manufacturer	Store in a dry place	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	

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.

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it