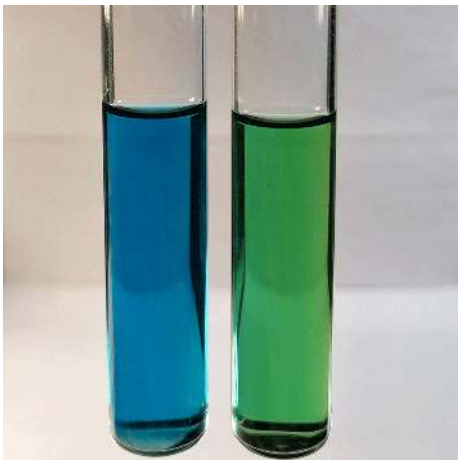
INSTRUCTIONS FOR USE

MALONATE BROTH

Dehydrated culture medium



Malonate Broth
From left: *E.aerogenes* malonate +, *E.coli*, malonate -

1 - INTENDED USE

In vitro diagnostic. For the differentiation of *Enterobacter* from *Escherichia* based on malonate utilisation.

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

Ammonium sulphate	2.000 g
Dipotassium phosphate	0.600 g
Monopotassium phosphate	0.400 g
Sodium chloride	2.000 g
Sodium malonate	3.000 g
Bromothymol blue	0.025 g
Glucose	0.250 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

The malonate test was introduced by Leifson (1933)[1] to help distinguish *Escherichia coli* from *'Klebsiella aerogenes*', and with these organisms he found a perfect correlation with the VP test.
An organism that simultaneously can utilize sodium malonate as a carbon source and ammonium sulphate as a nitrogen source produces an alkalinity due to the formation of sodium hydroxide. This results in an alkaline reaction which in a medium containing malonate, changes the indicator (bromothymol blue) from its original green colour to light blue or Prussian blue. Organisms which cannot utilize malonate and ammonium sulphate produce no colour change.
Malonate Broth is prepared according to a modification of the original formulation proposed by Leifson and does not include yeast extract as in Ewing's modified version.[2]

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 8.3 g in 1000 mL of cold distilled water, heat to dissolve, distribute into tubes and sterilise by autoclaving at 121 °C for 15 minutes. All glassware must be chemically cleaned and alkali-free.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	grey-green, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	green, limpid
Final pH at 20-25 °C	6.7 ± 0.1

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Malonate Broth	Dehydrated medium	4016852	500 g (60.2 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, sterile needles, incubator and laboratory equipment as required, test tubes, Erlenmeyer flasks, ancillary culture media and reagents for the identification of the colonies.

8 - SPECIMENS

The sample consists of bacterial cultures isolated from clinical samples or other materials, purified on Tryptic Soy Agar or blood agar or other suitable medium.

9 - TEST PROCEDURE

Inoculate the medium lightly with a loopful of a pure culture.
Incubate aerobically at 35-37°C for 24-48 hours. Observe the growth at the end of each period.

10 - READING AND INTERPRETATION

Examine the tubes for colour change.
Positive test: alkaline reaction, light blue to Prussian blue colour throughout the medium.
Negative test: no colour change of the medium
Bacterial genera in which the majority of species yields a positive alkaline reaction include: *Enterobacter, Klebsiella, Citrobacter.*
Bacterial genera in which the majority of species yields a negative reaction include: *Escherichia, Serratia, Salmonella, Morganella, Shigella Proteus, Edwardsiella, Providencia, Yersinia.*

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Consult the suitable references for the expected reactions for specific microbial species.[3,4]
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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.
Malonate positive strain: *E.aerogenes* ATCC 13048
Malonate negative strain: *E.coli* ATCC 25923

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 Malonate Broth is tested for specific performance characteristics by comparing the results with a previously approved Reference Batch. Samples are inoculated with cultures of malonate positive and malonate negative strains, grown for 18–24 h on Tryptic Soy Agar. The colour changes of tubed media are observed and recorded after 24 and 48-hours incubation at 35-37°C.
The following malonate positive strains turn the medium to blue: *K.pneumoniae* ATCC 23357, *E.aerogenes* ATCC 13048, *S.arizonae* ATCC 13314, *C.muytiensis* ATCC 51329, *E.cloacae* ATCC13047. The following malonate negative strains maintain unchanged the green colour of the medium: *E.coli* ATCC 25922, S.Typhimurium ATCC 14028, *S.marcescens* ATCC 8100, *P.rettgeri* ATCC 39944, *C.sakazakii* ATCC 29544.

13 - LIMITATIONS OF THE METHOD

- Some malonate negative organisms produce only a slight alkalinity that causes the results to be difficult to interpret. In case of doubt, compare with an uninoculated malonate tube. Any trace of a blue colour denotes a positive test at the end of 48 h incubation period. A final negative interpretation should be made until the tubes have been incubated for 48 h.[5]
- *E. coli* and *Klebsiella/Enterobacter* groups do not absolutely require yeast extract and glucose enrichments; however, when attempting to differentiate the *Salmonella* species from *S.arizonae* it is recommended that 1 g/L of yeast extract and 0,25 g/L of glucose be incorporated into the malonate medium, before autoclaving.[5]
- Even if the microbial colonies are differentiated on the basis malonate test, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification. If relevant, perform antimicrobial susceptibility testing.
- This culture medium is intended as an aid in the diagnosis of infectious disease; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

14 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, 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.
- 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 media 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.
- Notify Biolife Italiana Srl (complaint@biolifeitaliana.it) and the relevant Authorities of any serious incident occurring in connection with the use of the *in vitro* diagnostic.
- 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.

15 - 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 of prepared media and for the validation of the shelf life of the finished products, according to the type (tubes/bottles) and the storage method (temperature and packaging).

16 - REFERENCES

1. Leifson E. The Fermentation of Sodium Malonate as a Means of Differentiating Aerobacter and Escherichia. J Bacteriol. 1933 Sep; 26(3): 329–330.
2. Ewing WH, Davis BR, Reavis RW. Phenylalanine and Malonate Media and Their Use in Enteric Bacteriology. Public Health Lab. 1957; 15: 153-167.
3. Buchan BW et al Escherichia, Shigella and Salmonella. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
4. Forsythe SJ et al Klebisella and selected Enterobacterales. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
5. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/04
Revision 2	Removal of obsolete classification	2023/04

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

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