IFU

Instructions for Use

MALONATE BROTH

Cat. no. K36	Malonate Broth, 13x100mm Tube, 5ml	20 or 100 tubes/box

INTENDED USE

Hardy Diagnostics Malonate Broth is recommended for use in the differentiation of Enterobacteriaceae on the basis of malonate utilization.

SUMMARY

Malonate Broth was developed by Leifson in 1933.[7] Leifson's medium allowed for the differentiation of *Enterobacter* from *Escherichia* spp. based on malonate utilization. The medium contained ammonium sulfate as the sole nitrogen source, malonate as the sole carbon source, phosphates as buffers, and bromothymol blue as the pH indicator.

Hardy Diagnostics Malonate Broth follows a modified formula developed by Edward and Ewing in 1962.[8,9] The medium contains the same basal medium as Leifson's Malonate Broth with the addition of dextrose and yeast extract. Yeast extract serves as a source of vitamins while dextrose provides carbohydrates and a minimal amount of carbon. Incorporation of both ingredients initiate growth of organisms incapable of utilizing malonate or ammonium salt.

Organisms which simultaneously utilize malonate and ammonium sulfate produce sodium hydroxide which thereby results in an alkaline reaction and changes the indicator from its original green color to light blue or Prussian blue.[4] Organisms which cannot utilize malonate and ammonium sulfate and do not ferment dextrose produce no color change. Organisms which are malonate-negative but do ferment dextrose result in the development of a yellow color due to increased acidity in the medium.

FORMULA

Ingredients per liter of deionized water.*

Sodium Malonate	3.0gm
Ammonium Sulfate	2.0gm
Sodium Chloride	2.0gm
Yeast Extract	1.0gm
Dipotassium Phosphate	0.6gm
Monopotassium Phosphate	0.4gm
Dextrose	0.25gm
Bromothymol Blue	0.025gm

Final pH 6.7 +/- 0.2 at 25ºC.

*Adjusted and/or supplemented as required to meet performance criteria.

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-30ºC. away from direct light. Media should not be used if there are any signs of deterioration, discoloration, contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Specimen Collection: This medium is not intended for primary isolation of patient specimens. It should be used only with cultures of an isolated organism. This product is used in conjunction with other biochemical tests to identify cultures of an isolated organism.

Method of Use:

1. Using a light inoculum from an 18-24 hour pure culture, inoculate the tube.

2. Incubate the tube with loosened caps at 35ºC. in an aerobic atmosphere for 24-48 hours.

3. Observe for alkalization (blue color) at 24 and 48 hours.

INTERPRETATION OF RESULTS

A positive malonate test is indicated by the development of a blue color in the medium.

A negative malonate test is indicated by the media remaining green or turning yellow due to dextrose fermentation.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

The test organisms must be in pure culture and 18-24 hours old.

Some malonate-positive organisms produce only a slight alkalinity which renders difficulty in interpretation. Questionable results should be compared with an uninoculated malonate tube. Any trace of blue color is indicative of a positive test after 48 hours of incubation. Negative results should not be reported until a full 48 hour incubation period.[4]

Some malonate-negative strains ferment glucose only and produce a yellow color in the medium.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media, swabs, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Escherichia coli ATCC® 25922	A	24hr	35°C	Aerobic	Growth, broth remains green
Klebsiella pneumoniae ATCC® 13883	A	24hr	35°C	Aerobic	Growth, broth turns deep blue

* Refer to the document "Inoculation Procedures for Media QC" for more information.

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

Malonate Broth should appear clear, and bluish-green in color.

Escherichia coli (ATCC® 25922) growing in Malonate Broth (Cat. no. K36). Incubated aerobically for 24 hours at 35ºC.

Klebsiella pneumoniae (ATCC® 13883) growing in Malonate Broth (Cat. no. K36). Incubated aerobically for 24 hours at 35ºC.

Uninoculated tube of Malonate Broth (Cat. no. K36).

REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

2. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.

3. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.

4. MacFaddin, J.F. 1985. *Media for Isolation, Cultivation, Identification, Maintenance of Bacteria*, Vol. I. Williams & Wilkins, Baltimore, MD.

5. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.

6. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

7. Leifson. 1933. *J. Bacteriol.* ; 26:329-330.

8. Ewing, et al. 1957. *Public Health Lab*; 15:153-167.

9. Davis, et al. 1957. *Int. Bull. Bact. Nomencl. Taxon.*; 7:151-157.

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

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