

TECHNICAL DATA SHEET

Malonate Broth, Ewing Modified

Principle

Malonate broth is formulated by Leifson (1933) consist of yeast extract, ammonium sulphate, dipotassium phosphate, potassium dihydrogen phosphate, sodium chloride, sodium malonate, dextrose and bromothymol blue. The media is developed to differentiated *Enterobacter* form *Escherichia species* based on their ability to utilize malonate. The yeast extract provides nitrogen, carbon, vitamins and other essential elements. Ammonium salt is synthetic source of nitrogen. Phosphate acts as buffering agents. Sodium chloride maintains osmotic equilibrium. Dextrose is carbon source. Bromothymol blue is pH indicator dye. The medium, therefore, will support the growth of organisms that cannot utilize malonate or ammonium salt. The spontaneous alkalization produced by organisms is buffered by the phosphate system and neutralized by the acid produced by the fermentation of the small amount of dextrose. An alkaline reaction (blue color) is produced in this medium by organisms capable of utilizing malonate and ammonium sulfate. The alkali changes the color of the bromothymol blue indicator in the medium to light blue and finally to prussian blue. The color of the medium remains unchanged in the presence of an organism that cannot utilize these substances. Some malonate-negative strains produce a yellow color due to the fermentation of dextrose only, which results in increased acidity causing the pH indicator to change to yellow at a pH of 6.0. Also, some malonate-positive organisms produce only a slight alkalinity that causes the results to be difficult to interpret. Consequently, these tubes should be compared with an un-inoculated malonate tube.

Use: For the differentiation of members of Enterobacteriaceae on the basis of malonate utilization.

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Contents*

Ingredients	Gram/Litre
Yeast extract	1.000
Ammonium sulphate	2.000
Dipotassium phosphate	0.600
Potassium dihydrogen phosphate	0.400
Sodium chloride	2.000
Sodium malonate	3.000
Dextrose	0.250
Bromothymol blue	0.025
pH at 25°C	6.7 ±0.2

* Formula adjusted for optimum performance and parameters

Directions: Dissolve 9.28 grams in 1000 ml distilled water. Heat, if necessary, to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121 °C) for 15 min, Cool to 20-25°C and aseptically inoculate test sample.

Specimens' types analyzed

Clinical samples, food, water samples etc.

Precautions to be taken

These microbial media are intended for the in-vitro use only. All the handling, experiments, storage, and discarding should be performed with the help of skilled and knowledgeable technicians and as per the established guidelines. The material should be disposed only after proper sterilization by autoclaving. Please go through the MSDS of the media to avoid any accidents or in emergency.

Performance and Evaluation

The expected performance of the medium is liable to use as per the direction on the label when stored at optimum conditions and within expiry date.

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Quality Control

Appearance	Beige colored with green ting free flowing, homogeneous powder
Reaction of (0.93% w/v aqueous solution)	6.7 $\pm$ 0.2 at 25 °C
pH	6.50- 6.90
Color and clarity of ready medium	Blue green colored clear solution
Negative control	Performed using sterile distilled water

Different Microbial Response: Cultural characteristics observed after incubation at 25-30°C for 48-72 hours. Inoculum 50-100 CFU.

Organism	ATCC	Growth	Malonate utilization
<i>Klebsiella aerogenes</i>	13048	Luxuriant	Positive reaction, dark blue color
<i>Escherichia coli</i>	8739	Luxuriant	Negative reaction
<i>Salmonella typhimurium</i>	14028	Luxuriant	Negative reaction

Storage and Shelf Life: The product is highly hygroscopic; keep the container tightly closed at all times 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. Note: Sterilize media immediately after reconstitution.

Disposal: To avoid the contamination or propagation of any hazardous microbes the used, unusable or modified preparation of this product must be disposed after autoclaving after completion of task.

Reference

1. Ewing, W. H., Davis, B. R. and Reavis, R. W. (1957): Phenylalanine and malonate media and their use in enteric bacteriology. Public Health Lab., 15, 153-160.
2. Ewing, W. H., Mcwhorter, A. C., Escobar, M. R. and Lubin, A. H. (1965): Edwardsiella, a new genus of Enterobacteriaceae based on a new species, E. tarda. Internat. Bull. Bact. Nomen. Taxon., 15, 33-38
3. Leifson, E. (1933). The fermentation of sodium malonate as a means of differentiating Aerobacter and Escherichia. J. Bacteriol. 26: 329

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