

## CM 21139 - MALONATE BROTH EWING MODIFIED

### INTENDED USE

Differentiation of members of the Enterobacteriaceae on the basis of malonate utilization.

### PRODUCT SUMMARY AND EXPLANATION

Leifson developed a synthetic liquid medium, which differentiated Aerobacter (now Enterobacter) from Escherichia species based on their ability to utilize malonate where Enterobacter utilizes malonate and Escherichia does not. Ewing et al further modified this medium by the incorporation of yeast extract and dextrose. The medium, therefore, will support the growth of organisms that cannot utilize malonate or ammonium salt. Any spontaneous alkalization produced by such organisms is buffered by the phosphate system and counteracted 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.

### COMPOSITION

| Ingredients             | Gms / Ltr |
|-------------------------|-----------|
| Part I                  |           |
| Yeast extract           | 1.000     |
| Ammonium sulphate       | 2.000     |
| Dipotassium phosphate   | 0.600     |
| Monopotassium phosphate | 0.400     |
| Sodium chloride         | 2.000     |
| Bromothymol blue        | 0.025     |
| Dextrose                | 0.250     |
| Part II                 |           |
| Sodium malonate         | 3.000     |

### PRINCIPLE

An organism that can simultaneously utilize sodium malonate as its carbon source and ammonium sulfate as its nitrogen source produces alkalinity due to the formation of sodium hydroxide. 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. Therefore, these tubes should be compared with an un-inoculated malonate tube. The addition of yeast extract, a source of vitamins, and a relatively small amount of dextrose, a minimal carbon source, is included in Ewing's modification to stimulate the growth of some organisms.

### INSTRUCTION FOR USE

- Dissolve 9.28 grams in 1000 ml distilled water.
- Heat if necessary to dissolve the medium completely.
- Dispense and sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes.
- Avoid the addition of carbon and nitrogen from other sources.

### QUALITY CONTROL SPECIFICATIONS



Appearance of Powder : Light yellow to light green homogeneous free flowing powder.  
 Appearance of prepared medium : Bluish green coloured clear solution without any precipitate.  
 pH (at 25°C) : 6.7±0.2

## INTERPRETATION

Cultural characteristics observed after an incubation.

| Microorganism          | ATCC  | Inoculum (CFU/ml) | Growth    | Malonate Utilization                | Incubation Temperature | Incubation Period |
|------------------------|-------|-------------------|-----------|-------------------------------------|------------------------|-------------------|
| Enterobacter aerogenes | 13048 | 50-100            | Luxuriant | Positive reaction, dark blue colour | 35-37°C                | 18-48 Hours       |
| Escherichia coli       | 25922 | 50-100            | Luxuriant | Negative reaction                   | 35-37°C                | 18-48 Hours       |
| Klebsiella pneumoniae  | 13883 | 50-100            | Luxuriant | Positive reaction, dark blue colour | 35-37°C                | 18-48 Hours       |
| Salmonella Arizonae    | 13314 | 50-100            | Luxuriant | Positive reaction, dark blue colour | 35-37°C                | 18-48 Hours       |
| Salmonella Typhimurium | 14028 | 50-100            | Luxuriant | Negative reaction                   | 35-37°C                | 18-48 Hours       |

## PACKAGING:

In pack size of 100 gm bottles.

## STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

## DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

## REFERENCES

1. Leifson, 1933, J. Bact., 25:329.
2. Ewing W., Davis B. and Reavis R., 1957, Public Hlth. Lab., 15:153.
3. MacFaddin J., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.

