

## CM 20846 - K AGAR

### INTENDED USE

For isolation and cultivation of Alicyclobacillus in fruit juices in accordance with Official method of IFU.

### PRODUCT SUMMARY AND EXPLANATION

Alicyclobacillus are aerobic thermophilic non-pathogenic, spore forming bacteria that can survive the relatively mild pasteurization temperatures used for fruit juices and concentrates. Even very low numbers of Alicyclobacillus cause spoilage and off flavors in the beverages, damaging the brand. These bacteria are able to grow at pH values as low as 2.5 and also at elevated temperatures above 200°C. Their spores survive for long period in fruit concentrates and similar environments. *acidoterrestris* is the most commonly occurring species that produce taints in juice and similar products, however other species may also produce taints. Acidified environment are required to detect and isolate *A. acidoterrestris*, therefore, K-Agar is recommended for detection of taint producing Alicyclobacillus *acidoterrestris* as per IFU.

K-Agar (when incubated at 45°C) supports the growth of predominant *A. acidoterrestris* and limited growth of other species. If the sample is filterable, filter it through 0.45µm membrane after pretreatment. Filter aseptically and transfer one membrane on to K-Agar and other membrane on YSG Agar. Simultaneously, streak standard cultures of Alicyclobacillus *acidoterrestris* and Alicyclobacillus *acidocaldarius* for comparison. Incubate at 45±1°C for 2-5 days. Examine daily, for detailed procedure refer standard IFU method.

### COMPOSITION

| Ingredients   | Gms / Ltr |
|---------------|-----------|
| Yeast extract | 2.500     |
| Peptone       | 5.000     |
| Glucose       | 1.000     |
| Tween         | 1.000     |
| Agar          | 15.000    |

### PRINCIPLE

Peptone and yeast extract serve as a source of nitrogen, amino acids, vitamins, and other essential growth requirements. Glucose serves as a carbon source. Polysorbate 80 serves as an additional source of growth factor and fatty acid. The low acidic pH (3.7) of medium is obtained by addition of L-malic acid and is inhibitory to several bacterial species.

### INSTRUCTION FOR USE

- Dissolve 24.5 grams in 1000 ml distilled water.
- Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes.
- Cool to about 50°C and adjust pH 3.7 ± 0.1 with 25% L-malic acid.

### QUALITY CONTROL SPECIFICATIONS

- Appearance of Powder : Cream to yellow homogeneous free flowing powder.
- Appearance of prepared medium : Pale yellow coloured Clear to slightly opalescent gel forms in Petri plates.
- pH (at 25°C) : 3.7±0.1

### INTERPRETATION

Cultural characteristics observed after an incubation.



| Microorganism                    | ATCC  | Inoculum (CFU/ml) | Growth    | Recovery | Incubation Temperature | Incubation Period |
|----------------------------------|-------|-------------------|-----------|----------|------------------------|-------------------|
| Alicyclobacillus acidocaldarius  | 27009 | 50-100            | Poor-good | 10-40%   | 45- 46°C               | 2 -5 days         |
| Alicyclobacillus acidoterrestris | 49028 | 50-100            | Luxuriant | >=70%    | 45- 46°C               | 2 -5 days         |
| Alicyclobacillus acidocaldarius  | 43030 | 50-100            | Poor-good | 10-40%   | 45- 46°C               | 2 -5 days         |
| Escherichia coli                 | 25922 | >=10 <sup>3</sup> | Inhibited | 0%       | 45- 46°C               | 2 -5 days         |
| Staphylococcus aureus            | 25923 | >=10 <sup>3</sup> | Inhibited | 0%       | 45- 46°C               | 2 -5 days         |

#### PACKAGING:

Inpacksizeof100 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. IFU(2004). Standard IFU method No.12- Microbiological detection of Alicyclobacillus in fruit juices.  
International Federation of fruit juice producer, Paris
2. Matsubara et al. (2002). Alicyclobacillus acidiphilus sp. nov., a novel thermo-acidophilic-alicyclic fatty acid-containing bacterium isolated from acidic beverages. Int. J. Syst. Environment. Microbiol. 52, 1681-1685.
3. Baungart and Merve S. The Impact of Alicyclobacillus acidoterrestris on the quality of Juices and Soft Drinks Fruit processing 7: 251-254 (2000).



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|---------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------|
| <br>GMP<br>Good Manufacturing<br>Practices Certified | <br>Best Before                          | <br>QTY.<br>Quantity                  | <br>REF<br>Catalogue Number | <br>Manufacturer |
| <br>Temperature Unit                                 | <br>LOT/<br>B. NO.<br>Lot / Batch Number | <br>Consults Instructions<br>for Use | <br>QR<br>Code              |                                                                                                     |

NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

\*For LabUse Only

