

# **Technical Information**

# Nutrient Agar w/ Manganese

Product Code: DM 1931

Application: - Nutrient Agar with Manganese is used for promoting sporulation in Bacillus species.

### Composition\*\*

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Ingredients	Gms / Litre	
Beef extract	3.000	
Peptic digest of gelatin	5.000	
Manganese sulphate	0.030	
Agar	15.000	
Final pH ( at 25°C)	6.8±0.2	
**Formula adjusted, standardized to suit perfor	mance parameters	

### Principle & Interpretation

Nutrient Agar w/ Manganese, conventionally abbreviated as NAMn favours culture and sporulation of aerobic *Bacillus* species especially from canned foods.

Beef extract and pancreatic digest of gelatin provide necessary nutrients for growth of *Bacillus* species. Manganese is known to influence and enhance sporulation in *Bacillus* species (1-4). It has been reported that organisms recovered from spoilage of foods such as fruit drinks, tomatoes, acidified onions and other canned foods sporulate well aerobically on Nutrient Agar containing manganese (5).

Thermophilic bacteria such as *B. stearothermophillus* are capable to grow at 55-65°C while an incubation temperature of 30 to 35°C is favorable for culture and sporulation of mesophilic spore formers <sup>(5).</sup> This property is used to grow and therefore differentiate mesophilic and thermophilic spoilage bacteria. As recommended by APHA, in routine diagnosis for spoilage in canned foods, microbiological cultural procedures involve the use of primary recovery media and subculture media to identify spoilage bacteria and study its growth characteristics. Recovery media for aerobes generally include DTA (Dextrose Tryptone Agar) (DM1092) or DTB (Dextrose Tryptone Bromocresol Broth) (DM1122). Use of Cooked Meat Medium (DM1149) is recommended for recovery of anaerobic organisms. NAMn is widely used as subculture media for aerobes.

Nutrient Agar with Manganese supports growth and enhances sporulation by aerobic spore-formers and can be used primarily to differentiate mesophilic from thermophilic *Bacillus* species. When rod shaped aerobes in pure culture are isolated on DTA (or DTB) media (DM1092/ /DM1122) and sporulation is not evident, the isolates should be subcultured on Nutrient Agar with Manganese, at the temperature of initial isolation. After incubation for upto 10 days, if spore production has taken place, the spores are heat shocked to destroy all vegetative cells and cultured on Nutrient Agar w/ Manganese at both 30-35°C and 55°C. The temperature at which outgrowth occurs from the spore state indicates whether the isolate is an obligate mesophile (growth at 30 to 35°C), an obligate thermophile (growth at 55°C) or a facultative thermophile (growth at 30 to 35°C and at 55°C).

### Methodology

Suspend 23.03 grams of powder media in 1000 ml distilled water. Shake well & heat to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.





# **Quality Control**

### Physical Appearance

Cream to yellow homogeneous free flowing powder

#### Gelling

Firm, comparable with 1.5% Agar gel

### Colour and Clarity of prepared medium

Light amber coloured clear to slightly opalescent gel forms in Petri plates

#### Reaction

Reaction of 2.3% w/v aqueous solution at 25°C. pH: 6.8±0.2

#### pH range

6.6-7.0

### Cultural Response/Characteristics

DM 1931: Cultural characteristics observed after an incubation at 35-37°C for upto 5 days

Organism	Inoculum(CFU)	Growth	Recovery
Bacillus stearothermophilus ATCC 7953	50-100	luxuriant(incubated at55°C for upto 5 days	>=50%
Bacillus coagulans ATCC8038	50-100	luxuriant (with sporulation)	>=50%
Bacillus licheniformis ATCC9945a	50-100	luxuriant (with sporulation)	>=50%
Bacillus megaterium ATCC 9855	50-100	luxuriant (with sporulation)	>=50%
Bacillus polymyxa ATCC8526	50-100	luxuriant (with sporulation)	>=50%
Bacillus subtilis ATCC 6633	50-100	luxuriant (with sporulation)	>=50%

# Storage and Shelf Life

**Dried Media:** Store below 30°C in tightly closed container and use before expiry date as mentioned on the label. **Prepared Media:** 2-8<sup>0</sup> in sealable plastic bags for 2-5 days.

## **Further Reading**

- 1. Charney, J., Fisher, W. P. and Hegarty C. P., 1951, J. Bacteriol., 62:145.
- 2. Curran, H. R. and Evans F. R., 1954, J. Bacteriol., 67: 489.
- 3. Maunder D. T., 1970, "Examination of canned foods for microbial spoilage." Microbiology, Metal Div. R. and D, Continental Can Co., Inc., Oak Brook, III.
- 4. Penna T. C., Machoshvili I. A., Taqueda, M. E and Ferraz, C. A. 1998, PDA J. Pharm. Sci. Technol., 52 (5):198.
- 5. Downes F. P. and Ito K., (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., APHA, Washington, D.C.

### Disclaimer:

- User must ensure suitability of the product(s) in their application prior to use.
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