# PRODUCT SPECIFICATION SHEET

## M-Enterococcus Agar Base (DM333)

**Intended Use**

M-Enterococcus Agar Base (DM333) is recommended for isolation and enumeration of *Enterococci* in water, sewage, food and other materials by MF technique as well as direct plating of specimens.

**Product Summary and Explanation**

Enterococcus group is a subgroup of the fecal streptococci that includes *E. faecalis, E. faecium, E. gallinarum,* and *E. avium*. Enterococci are differentiated from other streptococci by their capability to grow in 6.5% sodium chloride, at pH 9.6 and at 10°C and 45°C.[1] The enterococcal portion of the fecal streptococcus group is a valuable bacterial indicator for determining the extent of fecal contamination of recreational surface waters.[1] Slanetz, Bent and Bartley[2] formulated M Enterococcus Agar for the enumeration of Enterococci by the membrane filter technique. Slanetz and Bartley[3] modified it by the addition of Triphenyl Tetrazolium Chloride (TTC). This modified medium proved to be a superior membrane filtration medium for the enumeration of enterococci.
Increased recovery and larger colonies were obtained by incubating the inoculated membranes on the agar surface instead of on pads saturated with liquid medium. This medium is highly selective. The membrane filtration method has the advantages of being simpler to perform, not requiring confirmation and permitting a direct count of enterococci in 48 hours. Burkwell and Hartman used polysorbate 80 (0.5 ml/l) and sodium carbonate (2 ml of a 10% aqueous solution per litre) to increase sensitivity for direct plating of foods and increasing colony size.[4] M-Enterococcus Agar is used for the detection of faecal *Streptococcus* and *Enterococcus* groups using the membrane filtration technique as per standard methods.[5]

**Principles of the Procedure**

M-Enterococcus Agar Base contains casein enzymic hydrolysate and papaic digest of soyabean meal which act as source of carbon, nitrogen and other essential growth nutrients. Yeast extract is the vitamin source and dextrose supplies carbon. Dipotassium phosphate acts as a buffer for the medium. Sodium azide is the selective agent to suppress the growth of gram-negative organisms. TTC Triphenyl tetrazolium chloride (TTC) is the dye which serves as a rapid indicator of bacterial growth. TTC is reduced to insoluble formazan inside the bacterial cells, resulting in the production of red colonies.

**Formula / Liter**

| Ingredients | Gms / Liter |
|---|---|
| Casein enzymic hydrolysate | 15.00 |
| Papaic digest of soyabean meal | 5.00 |
| Yeast extract | 5.00 |
| Dextrose | 2.00 |
| Dipotassium phosphate | 4.00 |
| Sodium azide | 0.40 |
| 2,3,5-Triphenyl tetrazolium chloride | 0.10 |
| Agar | 10.00 |
| Final pH : 7.2 ± 0.2 at 25°C | |
| Formula may be adjusted and/or supplemented as required to meet performance specifications | |

**Precautions**

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.
3. Sodium azide has a tendency to form explosive metal azides with plumbing materials. It is advisable to use enough water to flush off the disposables.

**Directions**

1. Suspend 41.5 grams of the medium in one litre of distilled water.
2. Heat to boiling to dissolve the medium completely.

3. DO NOT OVERHEAT OR AUTOCLAVE.
4. Add 0.5 ml polysorbate 80 and 2 ml of 10% aqueous solution of sodium carbonate, if desired.
5. Dispense into Petri plates.

**Quality Control Specifications**

| | |
|---|---|
| **Dehydrated Appearance** | Cream to yellow homogeneous free flowing powder |
| **Prepared Medium** | Light pink coloured clear to slightly opalescent gel forms in Petri plates |
| **Reaction of 4.15% Solution** | pH : 7.2 ± 0.2 at 25°C |
| **Gel Strength** | Firm, comparable with 1.0% Agar gel |

**Expected Cultural Response:** Cultural characteristics observed after an incubation at 35-37°C for 24 - 48 hours.

| Sr. No. | Organisms | Results to be achieved | | |
|---|---|---|---|---|
| | | Inoculum (CFU) | Growth | Colour of colony (on membrane) |
| 1. | *Escherichia coli ATCC 25922* | >=$10^3$ | inhibited | -- |
| 2. | *Enterococcus faecalis ATCC 29212* | 50-100 | good-luxuriant | pink - dark red (maroon) |

The organisms listed are the minimum that should be used for quality control testing.

**Test Procedure**

1. For filtration, choose a sample size so that 20-60 colonies will result.
2. Transfer the filter aseptically to agar medium, avoiding air bubbles beneath the membrane.
3. The medium can also be directly inoculated by streaking the specimen and incubating the plates at 35-37°C for 24-48 hours.
4. Incubate the plates at 35°C for 48 hours. After incubation, count all light and dark red colonies as Enterococci.
5. Refer to appropriate references for standard test procedures.

**Results**

Refer to appropriate references for interpretation of results.

**Storage**

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

**Expiration**

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

**Limitations of the Procedure**

1. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedure

**Packaging**

**Product Name : M-Enterococcus Agar Base**
**Product Code : DM333**
**Available Pack sizes : 500gm**

**References**


1. Eaton, Rice and Baird (ed). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
2. Slanetz, Bent and Bartley, 1955, Publ. Health. Rep., 70:67.
3. Slanetz and Bartley, 1957, J. Bact., 74:591.
4. Burkwell and Hartman, 1964, Appl. Microbiol., 12:18.
5. Eaton A. D., Clesceri L. S. and Greenberg A. W., (Eds.), 1998, Standard Methods for the Examination of Water and Wastewater, 20th Ed., APHA, Washington, D.C.


**Further Information**

For further information please contact your local MICROMASTER Representative.


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