



PRODUCT SPECIFICATION SHEET

Enterococcus Confirmatory Broth (DM104)

Intended Use

Enterococcus Confirmatory Broth (DM104) is recommended for confirming the presence of *Enterococci* in water supplies and other sources.

Product Summary and Explanation

The enterococcus group is a subgroup of the fecal streptococci that includes *Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus gallinarum*, and *Enterococcus avium*.⁽¹⁾ Enterococci are found as normal flora in the gastrointestinal tracts of humans and animals. Due to their resistance to antimicrobial agents to which other Streptococci are generally susceptible, they are becoming increasingly important agents of human diseases largely.⁽²⁾ Enterococci are differentiated from other streptococci by their ability to grow in 6.5% sodium chloride, at pH 9.6 and at 10°C and 45°C.⁽¹⁾ This ability to grow in the presence of variable amounts of sodium chloride is a test that has been used to characterize several bacteria, including the viridans Streptococci. The presence of intestinal enterococci, also known as fecal streptococci, is a valuable bacterial indicator for determining the extent of fecal contamination of recreational surface waters, especially when the contamination occurred a long time ago and the less resistant coliform bacteria, including *Escherichia coli*, may already be dead at the time of analysis.

Sandholzer and Winter⁽³⁾ formulated Enterococcus Confirmatory Broth for the detection of Enterococci in water supplies, swimming pools, sewage etc. This medium has the same formula as Enterococcus Confirmatory Agar (DM103) except agar, sodium chloride and Penicillin, which is used to detect Enterococci from crabmeat and oysters etc.

Principles of the Procedure

Enterococcus Confirmatory Broth contains casein enzymic hydrolysate, yeast extract, dextrose which provides essential growth nutrients for Enterococci. Penicillin has inhibitory effect on Staphylococci. Sodium azide inhibits gram-negative organisms. Sodium chloride provides the necessary ions to maintain the osmotic balance of the medium.

Formula / Liter

Ingredients	Gms / Liter
Casein enzymic hydrolysate	5.00
Yeast extract	5.00
Dextrose	5.00
Sodium azide	0.40
Sodium chloride	65.00
Methylene blue	0.01
Final pH : 8.0 ± 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 80.41 grams of the medium in one litre distilled water.
2. Heat if necessary to dissolve the medium completely.





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3. Dispense in 100 ml quantities in tubes.
4. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
5. Cool to room temperature and add 65 units of Penicillin to each 100 ml of broth prior to use.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow may have slight green tinge homogeneous free flowing powder
Prepared Medium	Yellow coloured, clear solution which acquires greenish tinge at the surface on standing
Reaction of 8.04% Solution	pH : 8.0 ± 0.2 at 25°C
Gel Strength	Not Applicable

Expected Cultural Response: Cultural characteristics observed after an incubation at 45°C for 18-24 hours.

Sr. No.	Organisms	Results to be achieved	
		Inoculum (CFU)	Growth
1.	<i>Escherichia coli ATCC 25922</i>	>=10 ³	good-luxuriant
2.	<i>Enterococcus faecalis ATCC 29212</i>	50-100	good-luxuriant

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

Test Procedure

Refer to appropriate references for standard test procedures.

Results

1. The positive presumptive tests are confirmed by inoculating from Enterococcus Presumptive Broth (DM105) to Enterococcus Confirmatory slant-broth combination prepared with an Azide Agar medium (Enterococcus Confirmatory Agar, DM103) overlaid with a Salt Azide Penicillin Broth (Enterococcus Confirmatory Broth, DM104).
2. A negative catalase test is considered confirmed positive evidence of the presence of Enterococci.
3. Single strength medium can be used for small inoculum.
4. Production of acid and turbidity in an azide presumptive broth when incubated at 45°C is considered positive presumptive evidence for the presence of Enterococci which is confirmed by inoculating in / on Confirmatory Broth / Agar (DM104, DM103).
5. 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 procedures.





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Packaging

Product Name : Enterococcus Confirmatory Broth

Product Code : DM104

Available Pack sizes : 500gm

References

1. Eaton A. D., Clesceri L. S. and Greenberg A. E., (Ed.), 1998, Standard Methods for the Examination of Water and Wastewater, 20th Ed., American Public Health Association, Washington, D.C.
2. Edwards M. S., Baker C. J., 1990, Principles and Practice of Infectious Diseases, 3rd Ed., pp 1554-1563, New York.
3. Sandholzer and Winter, 1946, Commercial Fisheries Leaflet T1a.

Further Information

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