PRODUCT SPECIFICATION SHEET

EC Broth (DM090)

Intended Use
EC Broth (DM090) is recommended for selective enumeration of faecal and non-faecal coliforms in water, wastewater and shell fish.

Product Summary and Explanation
EC Medium was originally described by Hajna and Perry and is used for the examination of water, milk, shellfish and other material for evidence of fecal pollution.[1] The use of this medium for the estimation of *E. coli* densities in seawater and shellfish was reported by Tennant et al.[2] This medium was later used by Fishbein and Surkiewicz to carry out *Escherichia coli* confirmatory tests. They used the EC confirmation test for recovery of *E. coli* from frozen foods and nut meats and reported that the test worked optimally when conducted at 45.5°C with incubation being limited to 24 hours.[3] EC Medium is recommended for confirmation of fecal coliform in Most Probable Number (MPN) procedure for the examination of water, wastewater and foods.[4,5] The procedure employing EC Medium provides information regarding the source of the coliform group (fecal or nonfecal) when used as a confirmatory test.[6] EC Broth should not be used for the direct isolation of coliforms since prior enrichment in a presumptive medium for optimal recovery of fecal coliforms is required.

Principles of the Procedure
EC broth contains casein enzymic hydrolysate which provides essential growth nutrients. Lactose is the fermentable carbohydrate for the growth of coliforms. Bile salts mixture inhibit gram-positive bacteria especially bacilli and faecal *Streptococci*. Phosphates control the pH during fermentation of lactose. The medium has a strong potassium phosphate buffering system to control the pH in the presence of considerable fermentative action. Sodium chloride maintains the osmotic balance of the medium.

Formula / Liter

Ingredients	Gms / Liter
Casein enzymic hydrolysate	20.00
Lactose	5.00
Bile salts mixture	1.50
Dipotassium phosphate	4.00
Monopotassium phosphate	1.50
Sodium chloride	5.00
Final pH: 6.9 ± 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.

Directions
1. Suspend 37 grams in 1000 ml distilled water.
2. Heat if necessary to dissolve the medium completely.
3. Dispense in test tubes containing inverted Durhams tube.
4. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
5. Adjust the concentration of medium in accordance with sample size.

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Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Yellow coloured, clear solution without any precipitate
Reaction of 3.7% Solution	pH : 6.9 ± 0.2 at 25°C
Gel Strength	Not Applicable

Expected Cultural Response : Cultural characteristics observed after an incubation at 44.5°C ± 0.2 for 24 hours.

Sr. No.	Organisms	Results to be achieved		
		Inoculum (CFU)	Growth	Gas formation
1.	*Bacillus subtilis ATCC 6633*	>=10 ^{3}	inhibited	
2.	*Escherichia coli ATCC 25922*	50-100	good- luxuriant	positive reaction
3.	*Enterobacter aerogenes ATCC 13048*	>=10 ^{3}	inhibited	
4.	*Enterococcus faecalis ATCC 29212*	>=10 ^{3}	inhibited	
5.	*Klebsiella pneumonia ATCC 13883*	50-100	good- luxuriant	positive reaction
6.	*Pseudomonas aeruginosa ATCC 27853*	50-100	fair to good	negative reaction

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

Test Procedure

When using sample more than 10 ml, the medium must be reconstituted at a concentration equivalent to that specified on the directions, once the sample is added, the working procedure is as follows:
Transfer a loopful of culture from all the tubes of Lauryl Sulphate Broth (DM347) showing gas formation within 24 hours and from all the tubes showing bacterial growth within 48 hours to EC Broth tubes. Within 30 minutes from the inoculum, place the tubes in a water bath and incubate at 44°C for 24 hours. Consider the growth showing gas production as positive.

Results

1. Gas production in a fermentation tube within 24 hour or less is a presumptive evidence of the presence of coliform bacteria. This medium can be used at 37°C for the detection of coliform organisms or at 44.5°C for the isolation of *Escherichia coli* from water and shellfish) or 45.5°C for foods.
2. Calculate the density of the faecal coliform organisms by using MPN tables.

Gas formation at 44.5°C or 45.5°C (and 37°C)	Escherichia coli, possibly also other coliforms
Gas formation at 37°C	Coliform bacteria without *Escherichia coli*

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

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1. False-negative reactions in recovering coliforms from water supplies can occur due to low pH, refrigeration and use of bactericidal or bacteriostatic agents.
2. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
3. Consult appropriate texts for detailed information and recommended procedures.

Packaging
Product Name : EC Broth
Product Code : DM090
Available Pack sizes : 100gm/ 500gm

References

1. Hajna and Perry. 1943. Am. J. Public Health *33*:550.
2. Tennant, Reid, Rockwell and Bynoe. 1961. Can. J. Microbiol. *1*:733.
3. Fishbein and Surkiewicz. 1964. Appl. Microbiol. *12*:127.
4. 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.
5. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
6. Wehr and Frank (ed.). 2004. Standard methods for the examination of dairy product, online. American Public Health Association, Washington, D.C.

Further Information

For further information please contact your local MICROMASTER Representative.

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