



## PRODUCT SPECIFICATION SHEET

### EE Broth, Mossel (DM616)

#### Intended Use

EE Broth, Mossel (DM616) is recommended for selective enrichment of *Enterobacteriaceae* in the bacteriological examination of foods.

#### Product Summary and Explanation

The *Enterobacteriaceae* family consists of *Salmonella*, *Shigella* and other enteric pathogens. These organisms find entry into the food system through faecally contaminated water. The enumeration of *Enterobacteriaceae* is of great concern in monitoring the sanitary condition of food. Majority of these organisms may be eliminated under the stringent food processing parameters. But some of these organisms may become sublethally injured during the changes in pH, exposure to steam or heat, preservatives, sanitizers and other unfavourable conditions. <sup>(1, 3)</sup> Therefore the important aspect of food monitoring depends upon the identification and enumeration of these injured cells, after resuscitation. EE Broth Mossel Enrichment is prepared according to the formula of Mossel, Visser and Cornelissen. <sup>(1, 2)</sup> The formula contains dextrose to facilitate growth of most *Enterobacteriaceae* particularly in food samples, thus ensuring the detection of *Salmonella* and other lactose-negative organisms. Nuisance organisms are suppressed by the addition of Ox Bile and Brilliant Green. EE Broth Mossel Enrichment should be used as an enrichment broth, followed by a selective medium; e.g., Violet Red Bile Agar. <sup>(4)</sup> EE Broth Mossel Enrichment is used to detect and enumerate *Enterobacteriaceae* found per millilitre or per gram of test sample of food when performing the Most Probable Number (MPN) technique with pre-enrichment. <sup>(5, 6)</sup> EE Broth Mossel Enrichment is listed in the *USP* as one of the recommended media for the isolation of bile-tolerant gram-negative bacteria from nonsterile pharmaceutical products. <sup>(7)</sup>

#### Principles of the Procedure

Peptic digest of animal tissue provides nitrogen, vitamins and amino acids. Dextrose is a carbon source which provides essential nutrients required for the growth of most of the members of *Enterobacteriaceae*. Brilliant green and ox bile, purified inhibit growth of gram-positive bacteria. Lactose-negative, anaerogenic lactose-positive or late lactose-fermenting *Enterobacteriaceae* are often missed by the standard coli-aerogenes test. Dextrose is replaced by lactose in this medium to overcome this problem. Disodium and monopotassium phosphates form the buffering system of the medium. Prior to enrichment in EE Broth, the cells damaged while drying or low pH are resuscitated in well-aerated Tryptone Soya Broth (DM277) for 2 hours at 25°C. The resuscitation procedure is recommended for dried foods, animal feeds and semi-preserved foods. EE Broth is an enrichment broth and should be used in conjunction with Violet Red Bile Glucose Agar (DM287). Subcultures must be made onto lactose differential media such as MacConkey Agar (M081), Deoxycholate Citrate Agar (DM577) or Brilliant Green Agar (DM044) for the detection of lactose negative or delayed lactose fermenters. This is used to inoculate MPN tubes prepared using EE Broth. Inoculate a loopful from these tubes onto Violet Red Bile Glucose Agar (M581) after an initial incubation at 35-37°C for 24 hours. Typical pink colonies from DM287 are subcultured for biochemical confirmation by oxidase and fermentation reactions. Decimal dilutions of the food homogenate are used if the expected counts are high or else initial suspension is used.

#### Formula / Liter

Ingredients	Gms / Liter
Peptic digest of animal tissue	10.00
Dextrose	5.00
Disodium phosphate	6.45
Monopotassium phosphate	2.00
Ox bile, purified	20.00





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Brilliant green	0.0135
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.

### Directions

1. Suspend 43.46 grams of the medium in one liter of distilled water.
2. Mix well and dispense 120 ml amounts in 250 ml flasks or 9 ml amounts in tubes.
3. Stopper with cotton plugs or loose fitting caps.
4. Heat in free flowing steam or boiling water for 30 minutes.
5. DO NOT AUTOCLAVE. Avoid overheating of the medium.

### Quality Control Specifications

Dehydrated Appearance	Light yellow to greenish yellow homogeneous free flowing powder
Prepared Medium	Emerald green coloured, clear solution without any precipitate
Reaction of 4.35% Solution	pH : 7.2 ± 0.2 at 25°C
Gel Strength	Not Applicable

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

Sr. No.	Organisms	Results to be achieved		
		Inoculum (CFU)	Growth	Acid
1.	<i>Escherichia coli ATCC 8739</i>	50 -100	good-luxuriant	positive reaction, yellow colour
2.	<i>Pseudomonas aeruginosa ATCC 9027</i>	50 -100	good-luxuriant	positive reaction, yellow colour
3.	<i>Escherichia coli ATCC 25922</i>	50-100	good-luxuriant	positive reaction, yellow colour
4.	<i>Escherichia coli NCTC 9002</i>	50 -100	good-luxuriant	positive reaction, yellow colour
5.	<i>Pseudomonas aeruginosa ATCC 27853</i>	50 -100	good-luxuriant	positive reaction, yellow colour
6.	<i>Enterobacter aerogenes ATCC 14028</i>	50 -100	good-luxuriant	positive reaction, yellow colour
7.	<i>Proteus mirabilis ATCC 25933</i>	50 -100	good-luxuriant	positive reaction, yellow colour
8.	<i>Salmonella Enteritidis ATCC 13076</i>	50 -100	good-luxuriant	positive reaction, yellow colour
9.	<i>Shigella boydii ATCC 12030</i>	50 -100	good-luxuriant	negative reaction

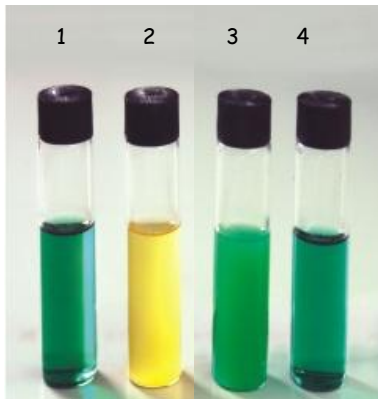




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10.	<i>Staphylococcus aureus</i> ATCC 25923	$\geq 10^3$	Inhibited	--
11.	<i>Staphylococcus aureus</i> ATCC 6538	$\geq 10^3$	Inhibited	--

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



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1. Control
2. *Escherichia coli* ATCC 25922
3. *Shigella boydii* ATCC 12030
4. *Staphylococcus aureus* ATCC 25923

### Test Procedure

#### 1. For food samples

Refer to appropriate references for details on sample collection, preparation and test methods for performing MPN technique with enrichment using EE Broth Mossel.

#### 2. For pharmaceutical samples

Refer to the *USP* for details on sample collection, preparation for testing of nonsterile products and tests for isolating *Enterobacteriaceae* using EE Broth Mossel.

### Results

1. A positive reaction results in acid production which causes the color of EE Broth Mossel to become yellow.
2. A negative reaction results in no color change and the medium remains green.

### Storage

Store the sealed bottle containing the dehydrated medium at 2 - 8°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. Some strains may grow poorly or fail to grow on this medium.
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.





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## Packaging

Product Name : EE Broth, Mossel.

Product Code : DM616

Available Pack sizes : 100gm / 500gm

## References

1. Mossel D. A. A., and Harrewijn G. A., 1972, Alimenta II, 29-30
2. Mossel D. A. A., Vissar M. and Cornellsen A. M. R., 1963, J. Appl. Bacteriol., 26(3):444.
3. Hartman, P. A., and S. A. Minnich. 1981. Automation for rapid identification of salmonellae in foods. J. Food Prot. 44:385-386.
4. Van Schothorst M. et al, 1966, Vet Med., 13(3):273.
5. International Organization for Standardization. 2004 Microbiology of food and animal feeding stuffs - horizontal methods for the detection and enumeration of *Enterobacteriaceae* - Part 1: Detection and enumeration by MPN technique with pre-enrichment. ISO 21528-1, 1st ed., 2004-08-15. International Organization for Standardization, Geneva, Switzerland.
6. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

## Further Information

For further information please contact your local MICROMASTER Representative.



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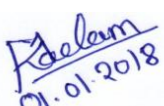
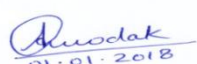

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