



## EE Broth-Mossel

Liquid medium for the cultivation and selective enrichment of Enterobacteriaceae from different types of samples, according to USP/EP/JP.

### DESCRIPTION

Enterobacteriaceae Enrichment Broth-Mossel is a selective medium used for the detection of bile-tolerant Gram-negative bacteria in food and other materials of sanitary importance.

This medium complies with the recommendations of the harmonized method in the United States Pharmacopoeia (USP), European Pharmacopoeia (EP) and Japanese Pharmacopoeia (JP) for the microbiological examination of nonsterile products.

TYPICAL FORMULA	(g/l)
Pancreatic Digest of Gelatin	10.0
Glucose Monohydrate	5.0
Dehydrated Ox Bile	20.0
Potassium Dihydrogen Phosphate	2.0
Disodium Hydrogen Phosphate, Anhydrous	6.4*
Brilliant Green	0.015
Final pH 7.2 ± 0.2 at 25°C	

\* Equivalent to 8.0 g of Disodium Hydrogen Phosphate Dihydrate.

### METHOD PRINCIPLE

Pancreatic digest of gelatin provides amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Glucose is the fermentable carbohydrate. Ox bile and brilliant green are selective agents effective against Gram-positive cocci. Potassium phosphate and sodium phosphate act as buffer.

### PREPARATION

Dehydrated medium Suspend 43.4 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil shaking frequently until completely dissolved. DO NOT AUTOCLAVE.

### TEST PROCEDURE

As in the Pharmacopoeia, prepare the sample using a 1 in 10 dilution of not less than 1 g of the product to be examined by choosing Tryptic Soy Broth (ref. 24513 or 452080) as diluent and incubate at 20-25°C for 2-5 hour to resuscitate bacteria.

For qualitative test (test for absence), transfer the volume of the pre-enrichment broth corresponding to 1 g of the product to be examined to EE Broth-Mossel.

For quantitative test, enumerate Enterobacteriaceae found per milliliter or per gram of test sample by using the Most Probable Number (MPN) technique. Use the volume of the pre-enrichment broth containing 0.1 g, 0.01 g and 0.001 g (or 0.1 ml, 0.01 ml and 0.001 ml) of the product to be examined to inoculate EE Broth-Mossel.

For both types of test incubate EE Broth-Mossel at 30-35°C for 24-48 h and continue analysis by subculturing on Violet Red Bile Glucose Agar (ref. 11184). Incubate plates aerobically at 30-35°C for 18-24 hours.

### INTERPRETING RESULTS

Turbidity of EE Broth-Mossel indicates microbial growth; acid production causes a color change of the medium to yellow.

No growth of colonies on Violet Red Bile Glucose Agar is reported as absence of bile-tolerant Gram-negative bacteria.

Growth of colonies constitutes a positive result and the probable number of bacteria is determined from the table below.

#### MPN Table.

Results for each quantity of product			Probable number of bacteria per gram or per milliliter of product
0.1 g or 0.1 ml	0.01 g or 0.01 ml	0.001 g or 0.001 ml	
+	+	+	>10 <sup>3</sup>
+	+	—	10 <sup>3</sup> - 10 <sup>2</sup>
+	—	—	10 <sup>2</sup> - 10
—	—	—	<10

### APPEARANCE

Dehydrated medium: free-flowing, homogeneous, light beige to light green.

Prepared medium: clear, green.

### STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store bottles and prepared plates at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

### SHELF LIFE

Dehydrated medium: 4 years.

Medium in tubes/bottles: 1 year.

**QUALITY CONTROL**

The medium is inoculated with the microbial strains indicated in the QC table.

Inoculum for productivity: ≤100 CFU.

Inoculum for selectivity: >100 CFU.

Incubation conditions: 18-24 h at 30-35°C (Pharmacopoeia growth promotion).

**QC Table.**

Microorganism		Specification
<i>Escherichia coli</i>	ATCC® 8739	Good growth
<i>Pseudomonas aeruginosa</i>	ATCC® 9027	Good growth
<i>Staphylococcus aureus</i>	ATCC® 6538	Inhibition

**WARNING AND PRECAUTIONS**

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use. The product is intended for professional use only and must be used by properly trained operators.

**DISPOSAL OF WASTE**









Disposal of waste must be carried out according to national and local regulations in force.

**BIBLIOGRAPHY**

1. EN ISO 11133:2014. Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media.
2. European Pharmacopoeia 6.5 (2009) 2.6.13. Microbiological examination of non-sterile products: Test for specified microorganisms.
3. United States Pharmacopoeia 32 NF 27 (2009) <62> Microbiological examination of non-sterile products: Test for specified microorganisms.
4. Japanese Pharmacopoeia 4.05 (2008) Microbiological examination of non-sterile products: Test for specified microorganisms.
5. ISO 21528-1:2004. Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of Enterobacteriaceae – Detection and enumeration by MPN technique with pre-enrichment.
6. ISO 21528-2:2004. Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of Enterobacteriaceae – Colony count method.
7. Davidson, Roth, and Gambrel-Lenarz (2004) In Wehr and Frank (ed.) Standard methods for the microbiological examination of dairy products, 17<sup>th</sup> ed. American Public Health Association, Washington, D.C.
8. Kornacki and Johnson (2001) In Downes and Ito (ed.) Compendium of methods for the microbiological examination of foods, 4<sup>th</sup> ed. American Public Health Association, Washington D.C.
9. Mossel, Vissar, and Cornellisen (1963) J. Appl. Bacteriol. 26:444.

PRESENTATION		Contents	Ref.
EE Broth-Mossel	Tubes	20 x 10 ml tubes	24096
EE Broth-Mossel	Bottles (screw cap)	6 x 100 ml bottles	402480
EE Broth-Mossel	Bottles (flip-off cap)	25 x 100 ml bottles	453080
EE Broth-Mossel	Bottles (perforable cap)	6 x 100 ml bottles	495000
EE Broth-Mossel	Dehydrated medium	500 g of powder	610017
EE Broth-Mossel	Dehydrated medium	100 g of powder	620017

**TABLE OF SYMBOLS**

<b>LOT</b> Batch code	 Keep away from sunlight	 Manufacturer	 Use by	 Fragile, handle with care
<b>REF</b> Catalogue number	 Temperature limitation	 Contains sufficient for <n> tests	 Caution, consult Instruction For Use	 Do not reuse



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