

## EE Broth

For the detection and enumeration of Enterobacteriaceae by the MPN method.

### Practical information

Applications	Categories
Non selective enumeration	Enterobacteria
Detection	Enterobacteria

Industry: Food

Regulations: ISO 11133

### Principles and uses

EE Broth is a medium recommended to detect and enumerate Enterobacteriaceae by the MPN method (Most probable number method).

Pancreatic digest of gelatin and glucose are the nitrogen and energy sources. Dehydrated ox bile and Brilliant green inhibit gram positive bacteria and most gram negative bacteria. Sodium phosphate and potassium phosphate act as a buffer system.

ISO 21528-1:2004 outlines a method, including pre-enrichment, for the detection of Enterobacteriaceae. It can be applied to products for human consumption and the feeding of animals, as well as environmental samples in the area of food production and food handling. This method is used when the microorganisms sought are expected to need resuscitation before enrichment, and when the number sought is expected to be in the range 1 to 100 per millilitre or per gram of test sample.

### Formula in g/L

Brilliant green	0,0135	Disodium phosphate	6,45
Gelatin pancreatic digest	10	Glucose monohydrate	5
Monopotassium phosphate	2	Dehydrated Ox Bile	20

### Preparation

Suspend 43,5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. AVOID OVERHEATING. DO NOT STERILIZE. Cool immediately under tap water without contaminating the medium. Dispense into appropriate containers.

### Instructions for use

- Dilute the sample (1:10) in buffered peptone water (BPW).
- Incubate the initial suspension at 37 °C for 18±2 hours.
- Transfer 1 ml of this suspension to 10 ml of EE Broth enrichment medium.
- Incubate at 37 °C for 24±2 hours.
- Inoculate on a selective solid medium and incubate the plate at 37° C for 24±2 hours.
- Confirmation and counting.

### Quality control

Solubility	Appearance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25°C)
w/o rests	Fine powder	Light green	Green	7,2±0,2

### Microbiological test

According ISO 11133:

Incubation conditions: (37±1 °C / 24±2 h).

Inoculation conditions: Productivity qualitative (<100 CFU) / Selectivity (10<sup>4</sup>-10<sup>6</sup> CFU) / Specificity (10<sup>3</sup>-10<sup>4</sup> CFU).

Microrganisms	Specification	Characteristic reaction
Escherichia coli ATCC 8739 + Enterococcus faecalis ATCC 19433	>= 10 colonies on VRBG	Pink to red colonies with or without precipitation halo
Escherichia coli ATCC 8739 + Enterococcus faecalis ATCC 29212	>= 10 colonies on VRBG	Pink to red colonies with or without precipitation halo
Salmonella typhimurium ATCC 14028 + Enterococcus faecalis ATCC 19433	>= 10 colonies on VRBG	Pink to red colonies with or without precipitation halo
Salmonella typhimurium ATCC 14028 + Enterococcus faecalis ATCC 29212	>= 10 colonies on VRBG	Pink to red colonies with or without precipitation halo
Enterococcus faecalis ATCC 19433	Total inhibition (0)	
Enterococcus faecalis ATCC 29212	Total inhibition (0)	

## Storage

Temp. Min.:2 °C

Temp. Max.:8 °C

## Bibliography

ISO 21528-1. 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.

Department of Health NHS Executive: The Caldicott Committee. Report on the review of patient identifiable information. London, December 1997.

The European Parliament and the Council of the European Union. Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. Official Journal of the European Union. L226.