

EVA BROTH (ETHYL VIOLET AZIDE, LITSKY)

CAT N°: 1230

Selective medium for the confirmation and detection of enterococci and as a detector of fecal contamination in water

FORMULA IN g/l

Peptone Mixture	20.00	Dipotassium Phosphate	2.70
Dextrose	5.00	Sodium Azide	0.40
Sodium Chloride	5.00	Ethyl Violet	0.0008
Monopotassium Phosphate	2.70		

Final pH 7.0 ± 0.2 at 25°C

PREPARATION

Suspend 35.8 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. Dispense 10 ml amounts into test tubes and sterilize in autoclave at 121°C for 15 minutes. It is recommended to use a large inoculum as the medium is very selective and is used in the second phase of confirmation. The prepared medium should be stored at 2-8°C. The color is greyish.

The dehydrated medium should be homogeneous, free-flowing and light beige in color. If there are any physical changes, discard the medium.

Caution: This medium is toxic if swallowed, inhaled or comes into contact with skin. Wear gloves and eye/face protection.

USES

EVA BROTH is a selective medium specific for the detection and confirmation of enterococci in water, other specimens and foods. It is an indication of fecal contamination. EVA Broth, in conjunction with Rothe Broth (Glucose Broth with Azide - Cat. 1238) as the presumptive medium, is used to enumerate fecal enterococci in water, soil and food products by the MPN technique.

The presence of intestinal enterococci is an indicator for faecal contamination, especially when the contamination occurred long ago and the less resistant coliform bacteria, including *Escherichia coli*, are already dead when the analysis is carried out.

Sodium azide and Ethyl violet inhibit all Gram positive bacilli and Gram positive cocci except enterococci and Gram negative bacteria. Peptone mixture provides nitrogen, vitamins, minerals and amino acids essential for growth. Dextrose is the fermentable carbohydrate providing carbon and energy. Potassium phosphates act as a buffer system, and Sodium chloride supplies essential electrolytes for transport and osmotic balance.

The tubes are inoculated with the appropriate dilutions in series of 3 tubes for each dilution and incubated at 35 ± 2°C for 24-48 hours. The appearance of turbidity and, eventually, the formation of a violet (purple) button of growth at the bottom of the tube are characteristics of enterococci growth.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 24-48 hours.

Microorganisms	Growth
<i>Escherichia coli</i> ATCC 25922	Inhibited
<i>Staphylococcus aureus</i> ATCC 25923	Inhibited
<i>Streptococcus pyogenes</i> ATCC 19615	Inhibited
<i>Enterococcus faecalis</i> ATCC 29212	Good
<i>Enterococcus faecalis</i> ATCC 19433	Good

BIBLIOGRAPHY

Litsky W. Mallmann W.L Fifield C.W. A.J.P.H 1953. 43. 873-879
Mallman and Seligman. 195 A.J.P.H 40:286.

STORAGE

Once opened keep powdered medium closed to avoid hydration.

