

Enterococcus Confirmatory Agar

Cat. 1018

For the confirmation of enterococci presence in water and other sources of sanitary interest.

Practical information

Applications	Categories
Selective isolation	Enterococci

Industry: Water

Principles and uses

Enterococcus Confirmatory Agar is used to confirm the presence of enterococci in water and other sources of sanitary interest.

 The presence of intestinal enterococci, also known as fecal streptococci, is an indicator for fecal contamination, especially when the contamination occurred a long time ago and the less resistant coliform bacteria, including *Escherichia coli*, may already be dead at the time of analysis.

Casein peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group essential for bacterial growth. Dextrose is the fermentable carbohydrate providing carbon and energy. Sodium azide inhibits Gram negative bacteria and Methylene blue is an inhibitor of some Gram positive bacteria. Methylene blue is also the pH indicator. Bacteriological agar is the solidifying agent.

To the prepared test tubes, in order to cover half of the slanted surface, aseptically add a volume of either Enterococcus Selective Broth (Cat.1204) or Enterococcus Confirmatory Broth (same formulation as this medium but without the agar). Using growth from KAA Presumptive Broth (Cat. 1209), inoculate both the surface and the broth in the Confirmatory Agar/Broth mixture tube.

Formula in g/L

Dextrose	5	Bacteriological agar	15
Casein peptone	5	Methylene blue	0,01
Sodium azide	0,4	Yeast extract	5

Preparation

Suspend 30,4 grams of the medium in one liter of distilled water. Dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into tubes and sterilize in autoclave at 121 °C for 15 minutes. Allow to cool in a slanted position in order to obtain butts of 1,5-2,0 cm depth.

Instructions for use

 Incubate at 35±2 °C for 18 hours and examine to detect the presence of small pinpoint colonies. Perform a Gram stain and observe under a microscope looking for large chains of ovoid cells. Immediately perform a catalase test by adding 5 ml of H₂O₂ to the tube in study. If there is no generation of gases (negative test), this constitutes the confirmation of enterococci in the sample.

Quality control

Solubility	Appearance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25°C)
w/o rests	Fine powder	Beige-blue	Greenish blue	8,0±0,2

Microbiological test

Incubation conditions: (35±2 °C / 18 h).

Microorganisms

Specification

Enterococcus faecalis ATCC 19433
Escherichia coli ATCC 25922
Enterococcus faecium ATCC 6057

Good growth
Total inhibition
Good growth

Storage

Temp. Min.:2 °C
Temp. Max.:25 °C

Bibliography

Winter and Sandholzer U.S Det. Interior Fishery, Leaflet 201 Part II, Nov. 1946
Ewing W.H. 1986. Edwards and Ewing's identification of Enterobacteriaceae 4th Edition.