

## ENTEROCOCCUS CONFIRMATORY AGAR

**CAT Nº: 1018**

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

### FORMULA IN g/l

Dextrose	5.00	Sodium Azide	0.40
Yeast Extract	5.00	Methylene Blue	0.01
Casein Peptone	5.00	Bacteriological Agar	15.00

**Final pH 8.0 ± 0.2 at 25°C**

### 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. The prepared medium should be stored at 2-8°C. The color is blue-greenish.

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

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

### 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 ConfirmatoryAgar/Broth mixture tube.

The tubes are incubated at 35 ± 2°C for 18 hours and are examined 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 H<sub>2</sub>O<sub>2</sub> to the tube in study. If there is no generation of gases (negative test), this constitutes the confirmation of enterococci in the sample.

## MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of  $35 \pm 2^\circ\text{C}$  and observed after 18-24 hours.

Microorganisms	Growth
<i>Escherichia coli</i> ATCC 25922	Inhibited
<i>Enterococcus faecalis</i> ATCC 19433	Good
<i>Enterococcus faecium</i> ATCC 6057	Good

## 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* 4<sup>th</sup> Edition.

## STORAGE

Once opened keep powdered medium closed to avoid hydration.

