82981 Esculin Iron Agar
NutriSelect® Plus

For the verification of enterococcal colonies on membrane filters through which water samples have been filtered and which have been incubated on M Enterococcus Agar, modified (Cat. No. 42702)

Composition:

Ingredients	Grams/Litre
Esculin	1.0
Ferric ammonium citrate	0.5
Agar	15.0

Final pH 7.1 +/- 0.2 at 25°C

Store dehydrated powder between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Protect from moisture and light by keeping container in a low humidity environment. Use before expiry date on the label.

Appearance(color):	Light yellow & Light Beige & Light Brown, free flowing powder
Gelling:	Firm, comparable with 1.5% Agar gel
Color and Clarity:	Medium amber coloured, clear to slightly opalescent gel forms in Petri plates

Directions:
Suspend 16.5 g in 1 litre distilled water. Heat to boiling with frequent stirring. Sterilize by autoclaving at 121°C for 15 minutes. Cool to 45°C and pour into sterile petri plates to a depth of 4-5 mm.

Principle and Interpretation:
Enterococcus species are a subgroup of fecal streptococci that includes *E. faecalis*, E. faecium, *E. gallinarum* and *E. avium* (1). Enterococci are differentiated from other *streptococci* by their ability to grow in 6.5% sodium chloride, at pH 9.6, and at 10°C and 45°C (1). The enterococci portion of the fecal streptococcus group is a valuable bacterial indicator for determining the extent of fecal contamination of recreational surface waters (1).
Detection and quantitation of Enterococci is necessary because gastroenteritis is associated with swimming in recreational water, which is dependent of enterococcal densities (2). Esculin Iron Agar is used in conjunction with M Enterococcus Agar, Modified, (42702) for verification of enterococcal colonies in fresh and marine recreational water, as recommended by APHA (3). Esculin in the medium is hydrolyzed by Enterococci to form esculetin and dextrose. Esculetin reacts with the iron salt (ferric ammonium citrate) and produces a dark brown to black complex, which appears around the colonies.

In the membrane filtration technique, two media, namely M-Enterococcus Agar, modified (42702) and Esculin Iron Agar (82981) are used in conjunction, where the former serves as a selective medium while the later confirms the identification of colonies on the basis of its ability to hydrolyze esculin. The membrane filter used to filter the test water sample is aseptically placed on M-Enterococcus Agar, modified (42702) and incubated at 40-42°C for 48 hours. After incubation the membrane is aseptically transferred to Esculin Iron Agar (82981) plate and incubated at 40-42°C for 20 minutes. After incubation count and record the number of pink to red colonies with black or reddish brown precipitate on the underside of the membrane. If required, magnifying glass or fluorescent lamp may be used for counting the visible colonies. Following formula is used for the final calculation (3).
Enterococci / 100 ml = No of enterococcal colonies Volume of sample filtered / X 100

Cultural characteristics observed after an incubation of 18-24 hrs at 40-42°C on M-Enterococcus Agar, modified (42702) and after 20 minutes at 40-42°C on Esculin Iron Agar (82981).

Organisms (ATCC/WDCM)	Growth	Color of colony	Esculin hydrolysis
Escherichia coli (25922/ 00013)	+/-	-	Negative reaction
*Enterococcus faecalis (*29212/00087)	++/+++	Pink to red	Positive reaction, brown to black precipitate around colonies

References:

1. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
2. Cabelli et al, 1979, Am. J. Public Health, 69:690.
3. Eaton A. D., Clesceri L. S. and Greenberg A. E., (Ed.), 1998, Standard Methods for the Examination of Water and Wastewater, 20th Ed., American Public Health Association, Washington, D.C..

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.