Also known as
m-Azide Agar; m-Enterococcus Agar; m-Slanetz Enterococcus Agar

Specification
Solid differential selective medium for the detection and enumeration of enterococci according to ISO standards.

Formula * in g/L
Tryptose 20.0
Yeast extract 5.0
Dextrose 2.0
Dipotassium phosphate 4.0
Sodium azide 0.4
Agar 12.0

Final pH 7.2 ±0.1 at 25 °C

* Adjusted and /or supplemented as required to meet performance criteria

Directions
Suspend 43.4 g in 1 l of distilled water and heat to boiling. Sterilize by autoclaving at 121 °C for 15 minutes. Cool down to 50 °C and add 10 ml/l of sterile TTC solution 1 % (Art. no. DSHB3074). Mix well and distribute into sterile plates immediately.

Description
This formulation, without TTC, allows sterilization in the autoclave without the development of a pink colour due to formazan which is formed as a result of the partial thermal-reduction of TTC. This modification is more tedious in its preparation but provides a colourless medium, making the results easier to read and the colonies are more sharply defined.

Technique
For the membrane filtration technique, take 100 ml of a well mixed water sample, and pass it through a sterile membrane filter. Then wash with 30 ml of sterile water to rinse the funnel.
Using sterile forceps, transfer the membrane aseptically to the culture medium contained in a Petri dish, making sure that the filter surface faces upwards. Close the lid and invert the plate. Incubate at 36 ±2 °C for 44 ±4 hours. The developed colonies that appear red or purple in colour must be considered as enterococci, since these bacteria reduce Triphenyltetrazolium-HCl to an insoluble formazan which is red in colour. The secondary or accompanying Gram negative bacteria are inhibited by sodium azide.
For food samples, from a decimal dilution bank of the sample, spread 0.1 ml of the dilutions onto the plated medium using a Drigalsky loop. Incubation and examination is then carried out in the same way as in the membrane filtration technique.
Note: the presence of enterococci must be confirmed with complementary biochemical tests.

Quality control
Incubation temperature: 36 °C ± 2.0 **Incubation time:** 44 ± 4 h

Inoculum: Practical range 100±20 CFU. min. 50 CFU (productivity)/ 10^4-10^6 CFU (selectivity). Membran filter method (or spiral plate method), according to ISO 11133:2014/Amd 1:2018.

Microorganism	Growth	Remarks
Escherichia coli ATCC® 25922	Inhibited	Selectivity
Enterococcus faecalis ATCC® 29212	Productivity > 0.50	Dark red colonies
Enterococcus faecalis ATCC® 19433	Productivity > 0.50	Dark red colonies
Staphylococcus aureus ATCC® 25923	Inhibited	Selectivity
Enterococcus faecium ATCC® 6057	Productivity > 0.50	Pink to red colonies

References
· ATLAS, R.M. and L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press. Boca Raton. Fla. USA.
· ISO 7899-2:2000 Standard. Water Quality. Detection and enumeration of enterococci by membrane filtration method.
. ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
· LACHICA, LV.F. and P.A. HARTMAN (1968) Two improved media for isolating and enumerating enterococci in certain frozen foods. J. appl. Bact. 31:151-156.
· SLANETZ, L.W. and BARTLEY, C.H. (1957) Numbers of enterococci in water, sewage and faeces determined by the membrane filter technique with an improved medium. J. Bact. 74:591-596.

Storage
For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 ºC to 30 ºC).