72678 Bile Esculin Azide Agar ISO 7899-2:2000

Selective medium for the isolation and presumptive identification of intestinal enterococci by Membrane filtration method.

Composition:

Ingredients	Grams/Litre
Tryptone	17.0
Ox Bile	10.0
Yeast Extract	5.0
Sodium Chloride	5.0
Peptone	3.0
Esculin	1.0
Ferric Ammonium Citrate	0.5
Sodium Azide	0.15
Bacteriological Agar	15.0
Final pH 7.1 +/- 0.1 at 25°C	

Store prepared media below 8°C, protected from direct light. Store dehydrated powder, in a dry place, in tightly-sealed containers at 2-25°C.

Appearance:	Faintly yellow, faintly beige to faintly brown colored, homogeneous, free flowing powder.
Gelling:	Firm
Color and Clarity:	Lightly yellow, light brown-yellow to light brown colored, clear to slightly turbid gel forms in petri plates.

Directions:
Suspend 56.6 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 into appropriate containers and sterilize in autoclave at 121ºC for 15 minutes. Overheating can cause darkening of the medium. If tubes are used, allow cooling in a slanted position

Principle and Interpretation:
Bile Esculin Azide Agar is a modification of Bile Esculin Agar. The difference is the addition of the inhibitor sodium azide and reduction of the bile. The medium is more selective but still allows the organisms to growth rapid and shows good recovery rate. The presence of intestinal enterococci is an indication of faecal contamination. Most coliform bacteria, including *Escherichia coli* are less resistant and may be damaged or death while enterococci is still alive.

Tryptone and peptone are the sources of nitrogen and essential growth factors. Yeast extract acts as well nitrogenous compounds and additionally the vitamin B_{12} complex. Sodium azide acts largely inhibits the growth of gram-negative bacteria while sparing enterococci, staphylococci and streptococci. Ox bile inhibits most gram positives but not enterococci. Enterococci hydrolyse esculin to esculetin and dextrose, which reacts with ferric citrate producing a brownish black precipitate around the colonies. Tolerance to bile and the ability to hydrolyze esculin is the traditional and reliable test for the identification of enterococci. (4). Sodium chloride maintains the osmotic balance of the medium and Agar is the solidifying agent.

Cultural characteristics after transfer of membrane from previously incubated on Slanez & Bartley medium (for *E. faecalis* and *E. faecium*) or TSA (for *E. coli*) for 2 hours at 44±0.5°C.

Organisms (ATCC/WDCM)	Characteristic reaction
Enterococcus faecalis (29212/00087)	Brown-black halo
Enterococcus faecalis (19433/00009)	Brown-black halo
Enterococcus faecium (6057/00010)	Brown-black halo
Escherichia coli (25922/00013)	Absence of brown-black halo

References:

1. ISO 7899-2:2000 Water quality -- Detection and enumeration of intestinal enterococci -- Part 2: Membrane filtration method
2. R.R. Facklam, M.D. Moody, Presumptive identification of Group D Streptococci: The bile-esculin test, Appl. Microbiol 20:245 (1970)
3. K.L. Ruoff, Streptococcus. In P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Yolken (eds), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C. (1995)
4. MacFaddin J.F., 1985, Media for Isolation-Cultivation-Identification-Maintainance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore

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.