Technical Data Sheet

Bile Esculin Azide Agar

Ordering number: 1.46321.0020

Bile esculin azide agar is used for counting colonies of enterococci in drinking water to confirm suspect colonies by Slanetz and Bartley Medium.

Ten settle plates each with a diameter of 90 mm are single-bagged in transparent, hydrogen peroxide impermeable sleeves (non-irradiated). The sleeves consist of polypropylene with a barrier of PE-EVOH-PE.

The composition of the medium conforms to the specifications of the drinking water regulations as per DIN EN ISO 7899-2.

Mode of Action

The esculin content of the bile esculin azide agar is hydrolyzed by the enterococci. The end product, 6,7-dihydroxycumarin, bonds with iron(III) ions to form a yellow-brown to black compound, which diffuses into the medium. The addition of azide and bile salts generally inhibits the accompanying gram-positive and gram-negative flora. The selectivity for enterococci is improved by increasing the incubation temperature to 44 °C instead of 36 °C.

Typical Composition

Casein Peptone	17 g/l
Peptone	3 g/l
Yeast Extract	5 g/l
Ox Bile	10 g/l
NaCl	5 g/l
Ammonium Iron(III) Citrate	0.5 g/l
Esculin	1 g/l
Sodium Azide	150 mg/l
Agar	15 g/l

The appearance of the medium is clear and yellowish. The pH value is in the range of 6.9-7.3. The medium can be adjusted and/or supplemented according to the performance criteria required.

Application and Interpretation

Each plate is provided with a label including a data matrix code for paperless plate identification. The code consists of a two-dimensional 20-digit serial number, which harbors the following information:

digits 1-3: here code 201 (corresponds to article 146321); digits 4-9: lot number; digits 10-14: batch specific individual number; digits 15-20: expiration date (YY/MM/DD).

Please check each agar plate before using it on sterility and pay attention to aseptic handling in order to avoid false positive results

As specified by DIN EN ISO 7899-2, after membrane filtration of the sample, the filter is placed on Slanetz and Bartley Medium (article number 146709) and incubated for 44 ± 4 h at 36 ± 2 °C. All smooth colonies with red, chestnut-brown or pink staining are counted. For confirmation the filter is placed on a preheated Bile Esculin Azide Agar and incubated for 2 hours at 44 ± 0.5 °C and then examined immediately. All colonies with yellow-brown or black staining in the surrounding medium are counted as intestinal enterococci.

No additional identification is planned in accordance with DIN EN ISO 7899-2.

In addition to the confirmation of esculin hydrolysis, the confirmation of the Lancefield antigen D is used for identification of enterococci. The reactions listed by Devriese *et al.* and Ellerbroek *et al*. can be recommended for phenotypic identification and differentiation of enterococci.

Storage and Shelf Life

The product can be used for sampling until the expiry date if stored upright, protected from light and properly sealed at +2 °C to +8 °C.

Condensation can be prevented by avoiding quick temperature shifts and mechanical stress.

The testing procedures as described on the CoA can be started up to the expiry date printed on the label.

Disposal

Please mind the respective regulations for the disposal of used culture medium (e.g. autoclave for 20 min at 121 °C, disinfect, incinerate etc.).

Quality Control

Control Strains	ATCC #	Inoculum	Incubation	Expected Results
Enterococcus faecalis	29212	100-1000	20-24 h at 35-37 °C	good growth; grey-whitish, very small colonies; nutrient medium dark-brown to black, also around single colonies
Enterococcus faecium	6057	100-1000	20-24 h at 35-37 °C	good growth; grey-whitish, very small colonies; nutrient medium dark-brown to black, also around single colonies
Streptococcus pyogenes	12344	100,000-1,000,000	20-24 h at 33-35 °C	no growth
Staphylococcus aureus	6538	100,000-1,000,000	20-24 h at 33-35 °C	growth inhibited; colorless needlestick colonies; nutrient medium unchanged
Escherichia coli	25922	100,000-1,000,000	20-24 h at 33-35 °C	growth inhibited; colorless colonies
Pseudomonas aeruginosa	27853	100,000-1,000,000	20-24 h at 33-35 °C	no growth

Please refer to the actual batch related Certificate of Analysis.

Literature

Devriese, L. A., Pot, B. and Collins, M. D. (1993): Phenotypic identification of the genus Enterococcus and differentiation of phylogenetically distinct enterococcal species and species groups. J. Appl. Bacteriol. 75: 399-408.

DIN EN ISO 7899-2 (2000): Water quality - Detection and enumeration of intestinal enterococci - Part 2: Membrane filtration method.

Ellerbroek, L. Wichmann-Schauer, H. und Mac, K.N. (2004): Methoden zur Identifizierung und Isolierung von Enterokokken und deren Resistenzbestimmung [methods for identification and isolation of enterococci and determining their resistance]. Berlin 2004 (BfR-Wissenschaft) ISSN 1614-3795 ISBN 3-931675-84-X: 98 Seiten.

Regulation on the quality of water for human consumption (drinking water regulations – TrinkwV), 03. May 2001.

Ordering Information

Product	Cat. No.	Pack size
Bile Esculin Azide Agar	1.46321.0020	20 x 90 mm plates
ReadyPlate™ 55 Slanetz and Bartley ISO 7899	1.46765.0020	20 x 55 mm plates
ReadyPlate™ 55 Kit Slanetz and Bartley ISO 7899	1.46766.0150	Kit (150 plates & 150 filters)
ReadyPlate™ Slanetz and Bartley Medium acc ISO 7899	1.46709.0020	20 x 90 mm plates
ReadyPlate™ Slanetz and Bartley Medium acc ISO 7899	1.46709.0100	100 x 90 mm plates
S-Pak Membrane Filters MCW 0.45µm 47mm white gridded	HAWG047S6	4 x 150 pcs
EZ-Pak Filters MCE 0.45µm 47mm white gridded	EZHAWG474	4 x 150 pcs

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