Kanamycin Esculine Azide Agar

For the isolation, differentiation and enumeration of enterococci in foodstuffs, water and other materials according to MOSSEL et al. (1978).

Kanamycin esculin azide agar is, unlike culture media containing bile which sometimes exhibit a fluctuating selectivity towards D-streptococci, always highly selective for this group of bacteria.

Mode of Action

Kanamycin and azide largely inhibit the accompanying bacterial flora. D-streptococci are, however, only slightly sensitive to these substances, so they can grow almost normal and hydrolyse the glucoside esculin to give glucose and esculetin. Esculetin forms an olive green to black complex with iron(III) ions.

Typical Composition (g/litre)

Peptones from casein 20.0; yeast extract 5.0; sodium chloride 5.0; sodium citrate 1.0; sodium azide 0.15; kanamycin sulfate 002; esculin 1.0; ammonium iron(III) citrate 0.5; agar-agar 15.0.

Preparation

Suspend 47.5 g/litre, autoclave (15 min at 121 °C), and pour plates.

■ **Do not overheat.**

pH: 7.1 ± 0.2 at 25 °C.

The plates are clear and brown-bluish.

Experimental Procedure and Evaluation

Inoculate by spreading the samples on the surface of the plates.

Incubation: up to 3 days at 35 °C or 42 °C aerobically. The higher temperature increases the selectivity of the medium.

Enterococci colonies are surrounded by a dark zone. Confirmatory tests, e.g. catalase test, glucose utilisation and growth at 45°C ± 1 °C, may be carried out.

Literature

BRANDL, E., ASPERGER, H., PFLEGER, F., u. IBEN, CH.: Zum Vorkommen von D-Streptokokken in Käse. **- Arch. Lebensmittelhyg., 36**; 18-22 (1985).

MOSSEL, D.A.A., BIJKER, P.G.H., a. EELDERING, J.: Streptokokken der Lancefield-Gruppe D in Lebensmitteln und Trinkwasser - Ihre Bedeutung, Erfassung und Bekämpfung. **- Arch. f. Lebensmittelhyg., 29**; 121-127 (1978).

Ordering Information

Product	Merck Cat. No.	Pack size
Kanamycin Esculine Azide Agar	1.05222.0500	500 g

Quality control (spiral plating method)

Test strains	Inoculum (cfu/ml)	Recovery rate (%)	Colour change to olivegreen-black
Enterococcus faecalis ATCC 11700	10^3-10^5	≥ 70	+
Enterococcus hirae ATCC 8043 8043	10^3-10^5	≥ 70	+
Enterococcus durans BFM* 11507	10^3-10^5	≥ 70	+
Staphylococcus aureus ATCC 6538	10^3-10^5	-	-
Bacillus cereus ATCC 11778	> 10^5	≤ 0.01	-
Escherichia coli ATCC 11775	> 10^5	≤ 0.01	-

* Bundesanstalt f. Milchforschung, Kiel, Germany

Enterococcus faecalis ATCC 29212

Streptococcus pyrogenes ATCC 19615