



Kanamycin Esculin Azide Agar Base

M510A

Kanamycin Esculin Azide Agar Base is used for isolation of Group D Streptococci in foodstuffs.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	20.000
Yeast extract	5.000
Sodium chloride	5.000
Sodium citrate	1.000
Esculin	1.000
Ferric ammonium citrate	0.500
Sodium azide	0.150
Agar	10.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 21.32 grams in 500 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add rehydrated contents of one vial of Kanamycin Sulphate Selective Supplement (FD146). Mix well before pouring into sterile petri plates.

Warning: Sodium azide has a tendency to form explosive metal azides with plumbing materials. It is advisable to use enough water to flush off the disposables.

Principle And Interpretation

Kanamycin Esculin Azide media are formulated as per Mossel et al (1,2) to detect Enterococci in food stuffs. Mossel et al (3) used it for the dip slide technique for bacteriological monitoring of foods.

Casein enzyme hydrolysate, yeast extract provides essential nutrients for Enterococci. Kanamycin sulphate and Sodium azide are the selective inhibitory components. Esculin and Ferric ammonium citrate together form indicator system to detect esculin - hydrolysing Streptococci forming black zones around the colonies.

Mossel et al (4) adopted the following procedure as - 1gm or 1ml mixed food is added to prechilled diluent (Tryptone water M463) and decimal dilutions are prepared. The decimal dilution are inoculated in Kanamycin Esculin Azide Broth (M776) and incubated at 35° for 16-24 hours.

If blackening of medium occurs, streaking is done on agar (M510A) and after incubation confirmatory tests are carried out.

Quality Control

Appearance

Light yellow to light brown coloured homogeneous free flowing powder

Gelling

Firm, comparable with 1.0% Agar gel.

Colour and Clarity of prepared medium

Medium amber coloured clear to slightly opalescent gel with purplish tinge forms in petri plates.

Reaction

Reaction of 4.26% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

M510A: Cultural characteristics observed after an incubation at 35-37°C or 42°C for 18-24 hours .

Organism	Inoculum (CFU)	Growth	Recovery	Esculin Hydrolysis
<i>Enterococcus bovis</i> ATCC 27960	50-100	luxuriant	>=50%	Positive reaction, blackening of medium around the colony
<i>Enterococcus faecium</i> ATCC 19434	50-100	luxuriant	>=50%	Positive reaction, blackening of medium around the colony
<i>Escherichia coli</i> ATCC 25922	>=10 ³	inhibited	0%	

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label.

Reference

1. Mossel D.A.A., Bijker, P.G.H. and Eelderink I., 1978, Arch. Lebensmittel - Hyg., 29:121.
2. Mossel D.A.A., et al., 1978, In : Streptococci., Skinner F.A. and Quesnel L. B. (ed.), SAB Symposium, series No.7, Academic Press, London.
3. Mossel D.A.A., et al, 1976, Lab. Practice, 25:393.
4. Mossel D.A.A., Harrenwijn G.A. and Elzebroek B.J.M., 1973, UNICEF Geneva.
5. Reuter E., 1985, Inter. J. Food Microbiol., 2:103.

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