

## TM 2141 - KANAMYCIN ESCULIN AZIDE AGAR

### INTENDED USE

For selective isolation and identification of group D Streptococci in foodstuff.

### PRODUCT SUMMARY AND EXPLANATION

Kanamycin Esculin Azide Agar has been used successfully for the isolation of glycopeptide-resistant Enterococci from clinical specimens and foods. There is no universal medium that will isolate all strains of Enterococci. Unless a presumptive count is acceptable all isolates should have their identity confirmed with further tests.

Enterococci may be considered an essential part of the autochthonous microflora of humans and animals. Faecal streptococci bearing the group D Lancefield antigens are grouped as Enterococci. Lancefield Group D-Streptococci constituting the faecal Streptococci are contaminants of various food commodities, especially those of animal origin. Kanamycin Esculin Azide Agar is formulated as per Mossel et al to detect Enterococci in foodstuffs. Mossel et al used it for the dip slide technique for bacteriological monitoring of foods.

### COMPOSITION

Ingredients	Gms / Ltr
Tryptone	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
Kanamycin sulphate	0.020
Agar	12.000

### PRINCIPLE

Tryptone and yeast extract provides essential nutrients for Enterococci. Kanamycin sulphate and sodium azide are the selective inhibitory components. Esculin and ferric ammonium citrate together forms the indicator system to detect esculin- hydrolyzing Streptococci, which form black zones around the colonies. The black zones are produced from the formation of black iron phenolic compounds derived from esculin-hydrolysis products and ferrous ions. Mossel et al described the following procedure-1gm or 1ml mixed food is added to 9 ml of pre-chilled diluent (Tryptone water) and decimal dilutions are prepared. The decimal dilutions are inoculated in Kanamycin Esculin Azide Broth and incubated at 35-37°C for 16-24 hours. If blackening of medium occurs, streaking is done on agar and after incubation confirmatory tests are carried out.

### INSTRUCTION FOR USE

- Dissolve 44.67 grams in 1000 ml purified/distilled water.
- Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes.
- Cool to 45-50°C. Dispense as desired.

### QUALITY CONTROL SPECIFICATIONS



**Appearance of Powder** : Cream to yellow w/greenish tinge homogeneous free flowing powder.  
**Appearance of prepared medium** : Medium amber coloured, clear to slightly opalescent gel with purplish tinge forms in Petri plates.  
**pH (at 25°C)** : 7.0±0.2

### INTERPRETATION

Cultural characteristics observed after an incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Esculin Hydrolysis	Incubation Temperature	Incubation Period
<i>Enterococcus bovis</i>	27960	50-100	Good-luxuriant	>=50%	Positive, blackening of medium around the colony	35-37°C	18-24 Hours
<i>Enterococcus faecium</i>	19434	50-100	Good-luxuriant	>=50%	Positive, blackening of medium around the colony	35-37°C	18-24 Hours
<i>Escherichia coli</i>	25922	>=10 <sup>3</sup>	Inhibited	0%	-	35-37°C	18-24 Hours
<i>Enterococcus faecalis</i>	29212	50-100	Good-luxuriant	>=50%	Positive, blackening of medium around the colony	35-37°C	18-24 Hours
<i>Staphylococcus aureus subsp. aureus</i>	25923	>=10 <sup>3</sup>	Inhibited	0%	-	35-37°C	18-24 Hours

### PACKAGING:

In pack size of 100 gm bottles.

### STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

**Product Deterioration:** Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

### DISPOSAL













After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

### REFERENCES

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.



2. Chadwick P. R., Brown D. F. J., Wilcox M. H. et al, 1997, Clin. Microbiol., Inf. 3. 559-563.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook Second Edition.
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
5. Mossel D. A. A., Bijker P. G. H. and Eelderink I., 1978, Arch. Lebensmittel - hyg., 29:121.
6. Mossel D. A.A. et al, 1978, In: `Streptococci., Skinner F. A. and Quesnel L. B. (Eds.), SAB Symposium, Series No.7, Academic Press, London.
7. Mossel D. A. A. et al, 1976, Lab. Practice, 25:393.
8. Mossel D. A. A., Harrenwijn G. A. and Elzebroek B. J. M., 1973, UNICEF, Geneva.
9. Reuter G., 1985, Inter. J. Food. Microbiol., 2.103-114.
10. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015. Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
11. Van den Braak N., Van Belkum A., Van Keulen. M et al, 1998, J. Clin. Microbiol., 36. 1927-1932.
12. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

 <b>GMP</b> Good Manufacturing Practices Certified	 <b>IVD</b> For In Vitro Diagnostic Use	 <b>QTY.</b> Quantity	 <b>LOT/B. NO.</b> Lot / Batch Number	 <b>REF</b> Catalogue Number	 <b>Manufacturer</b>
 <b>Temperature Unit</b>	 <b>EC REP</b> MedNet GmbH Barkstrasse 10, 48163 Maastricht, Germany	 <b>European Conformity</b>	 <b>QR Code</b>	 <b>Consults Instructions for Use</b>	 <b>Best Before</b>

**NOTE:** Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

**\*For Lab Use Only**  
**Revision: 08 Nov., 2019**