

## TM 673 – BILE ESCULIN AGAR W/ KANAMYCIN

### INTENDED USE

For selective isolation and presumptive identification of *Bacteroides fragilis* from mixed flora.

### PRODUCT SUMMARY AND EXPLANATION

Group D Streptococci possess the group D lipoteichoic acid antigen in their cell walls. Former Group D species, which are predominant normal inhabitants of the human gastrointestinal tract, are termed as faecal Streptococci or Enterococci. The unique ability of Enterococci to split esculin was reported by Meyer and Schonfeld. Enterococci and Group D Streptococci hydrolyse esculin to esculetin and dextrose, which reacts with ferric citrate producing brownish black precipitate. The use of esculin hydrolysis in identification of Enterococci was first cited by Rochaix. Bile Esculin Agar was originally formulated by Swan for the isolation and identification of Group D Streptococci from food. Facklam and Moody further reported that using Bile Esculin Agar, Group D Streptococci could be differentiated from non-Group D Streptococci. Bile Esculin Agar was also shown to aid differentiation of *Enterobacteriaceae*, *Klebsiella*, *Enterobacter*, *Serratia* from other *Enterobacteriaceae* genera on the basis of esculin hydrolysis. However, other tests such as salt tolerance should be performed for identifying Enterococci.

Bile Esculin Agar with Kanamycin is recommended for the selective isolation and presumptive identification of *Bacteroides fragilis* group of bacteria from mixed flora. This medium is a modification of the original formulation of Swan. In this medium kanamycin is added to an enriched Bile Esculin Agar, enriched with hemin and vitamin K1. Hemin and vitamin K1 enriches and enhances the growth of *Bacteroides* species. Kanamycin selectively promotes the growth of *Bacteroides fragilis* while inhibiting the growth of facultative anaerobic and aerobic gram-negative bacilli. Anaerobes that are incapable of hydrolyzing esculin do not form brown or black pigmented colonies on this medium. The plates should be reduced by keeping in anaerobic jar for 18-24 hours, just before incubation.

### COMPOSITION

Ingredients	Gms / Ltr
Gelatin peptone	5.000
Beef extract	3.000
Oxgall	20.000
Ferric citrate	0.500
Esculin	1.000
Kanamycin	0.100
Hemin	0.010
Vitamin K1	0.010
Agar	15.000

### PRINCIPLE

Gelatin peptone and meat peptone B serves as sources of carbon, nitrogen, amino acids, vitamins and essential growth nutrients. Bile and kanamycin inhibits most of the other accompanying bacteria. Esculin in the medium is hydrolyzed to esculetin and dextrose. Esculetin reacts with ferric citrate to form a dark brown or black complex, visualized as a zone of black precipitate around the colonies. Viridans Streptococci sometimes exhibit a weak positive reaction.

### INSTRUCTION FOR USE

- Dissolve 44.6 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.
- Mix well and pour into sterile Petri plates. DO NOT OVERHEAT.

### QUALITY CONTROL SPECIFICATIONS

<b>Appearance of Powder</b>	: Light yellow to brownish yellow homogeneous free flowing powder.
<b>Appearance of prepared medium</b>	: Amber coloured, clear to slightly opalescent gel with a bluish tinge forms in Petri plates.
<b>pH (at 25°C)</b>	: 7.1±0.2

### INTERPRETATION

Cultural characteristics observed after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Esculin hydrolysis	Incubation Temperature	Incubation Period
<i>Bacteroides fragilis</i>	25285	50-100	Luxuriant	≥70%	Positive reaction, blackening of medium around the colony	35-37°C	24-48 Hours
<i>Escherichia coli</i>	25922	50-100	None-poor	0-10%	Negative reaction	35-37°C	24-48 Hours
<i>Fusobacterium necrophorum</i>	25286	50-100	None-poor	0-10%	Negative reaction	35-37°C	24-48 Hours

### PACKAGING:

In pack size of 100 gm and 500 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

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 10. Rochaix, 1924, Comt. Rend. Soc. Biol., 90:771.  
 11. Murray P. R., Baron E. J., Jorgensen J. H., Pfaller M. A., Tenenbaum R. H., Tenenbaum R. H.,(Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.

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

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

**\*For Lab Use Only**  
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