

## TM 1883 -BILE ESCULIN AZIDE AGAR (ISO 7899-2: 2000)

### INTENDED USE

For isolation and presumptive identification of faecal *Streptococci*.

### PRODUCT SUMMARY AND EXPLANATION

Bile Esculin Azide Agar is a modification of Bile Esculin Agar developed by Isenberg and recommended as a selective and highly nutritive medium for *Streptococci*. This formula modifies Bile Esculin Agar by adding sodium azide and reducing the concentration of bile. The revised medium is more selective, but still provides rapid growth and efficient recovery of group D *Streptococci*.

### COMPOSITION

Ingredients	Gms / Ltr
Casein enzymatic hydrolysate	17.000
Agar	15.000
Oxgall	10.000
Yeast extract	5.000
Sodium chloride	5.000
Peptic digest of animal tissue	3.000
Esculin	1.000
Ferric ammonium citrate	0.500
Sodium azide	0.150

### PRINCIPLE

Bile Esculin Azide Agar contains casein enzymatic hydrolysate, peptic digest of animal tissue and beef extract which supply the essential nutrients for *Streptococci* like e.g. amino acids, other nitrogenous and carbonaceous compounds. Sodium chloride maintains the osmotic equilibrium of the medium. The inclusion of esculin allows for detection of esculin hydrolysis by the bacterial enzyme, esculinase. Esculin hydrolysis liberates esculetin and dextrose which in turn reacts with ferric citrate in the medium to produce black phenolic iron-complex giving esculinase-positive colonies a brown black halo. Selectivity is accomplished by the addition of oxgall and sodium azide. Oxgall inhibits the growth of most gram-positive cocci other than Enterococci and group D *Streptococci*, while sodium azide inhibits gram-negative bacteria that may be contained in some clinical samples and agar is the solidifying agent.

### INSTRUCTION FOR USE

- Dissolve 56.65 grams in 1000ml distilled water.
- Gently heat to boiling with gentle swirling and dissolve the medium completely.
- Sterilize by autoclaving at 15 psi (121°C) for 15 minutes.
- Cool at 45 – 50°C.
- Pour into sterile Petri plates.

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

### QUALITY CONTROL SPECIFICATIONS

**Appearance of Dehydrated powder** : Light yellow to brownish yellow homogeneous free flowing powder



**Appearance of Prepared medium** : Amber colored, clear to slightly opalescent gel with bluish tinge  
**pH (at 25°C)** : 7.2± 0.2

### INTERPRETATION

Cultural characteristics observed after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Esculin hydrolysis	Incubation Temperature	Incubation Period
<i>Enterococcus faecalis</i>	29212	50-100	Luxuriant	>=50%	+	35-37°C	18 – 24 Hours
<i>Staphylococcus aureus</i>	25923	50-100	Good	40-50%	-	35-37°C	18 – 24 Hours
<i>Proteus mirabilis</i>	25933	50-100	Good	40-50%	-	35-37°C	18 – 24 Hours
<i>Streptococcus pyogenes</i>	19615	50-100	None-Poor	<=10%	-	35-37°C	18 – 24 Hours
<i>Escherichia coli</i>	25922	≥1000	Inhibited	0%	-	35-37°C	18 – 24 Hours

+ = Positive reaction, Blackening of medium around the colony

- = Negative reaction

### PACKAGING

In 500 gm packaging size.

### STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers below 25°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 powder show evidence of microbial contamination, discoloration, drying, or other signs of deterioration.










### DISPOSAL

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

### REFERENCES

1. Isenberg, H. D. 1970. Clin. Lab. Forum.
2. Isenberg, H. D., D. Goldberg, and J. Sampson. 1970. Laboratory studies with a selective enterococcus medium. Appl. Microbiol. 20:433.
3. International Organisation for Standardisation (ISO), Water quality - Detection and enumeration of intestinal enterococci, Draft, ISO/DIS 7899 (1984).
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5. Koneman E. W., Allen S. D., Janda W. M., Schreckenberger P. C., Winn W. C. Jr., 1992, Colour Atlas and Textbook of Diagnostic Microbiology, 4th Ed., J. B. Lippincott Company
6. Meyer and Schonfeld, 1926, Zentralbl. Bakteriol, Parasitenk. Infektionskr. Hyg. Abt. Orig. 99:402.
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9. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
10. ISO NORMATIVE 7899-2. Water quality -- Detection and enumeration of intestinal enterococci -- Part 2: Membrane filtration method



 GMP Good Manufacturing Practices Certified	 Best Before	 Quantity	 Catalogue Number	 Manufacturer
 Temperature Unit	 Lot / Batch Number	 Consults Instructions for Use	 QR Code	

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

**\*For Lab Use Only**

**Revision: 05<sup>th</sup> Oct. 2019**