

## **Product Data Sheet**

### **BILE ESCULIN AGAR BASE**

**Product No.** GB-DCM-00087-1A

### **INTENDED USE**

For differential & presumptive identification of group D Streptococci from food & pharma products.

### **PRODUCT SUMMARY**

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 Base with added supplements is recommended for selective isolation and presumptive identification of group D streptococci from food and pharmaceutical products. Esculin when added as a supplement 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. If the media is dispensed in tubes in the form of slants, a positive reaction is indicated by blackening of more than half of the slant within 24-48 hours. If blackening is totally absent or if less than half of the slant is blackened within 24-48 hours, the test is negative. Viridans Streptococci sometimes exhibit a weak positive reaction. Also, Leuconostoc, Pediococcus, Lactococcus species causing human infections give a positive bile esculin test. To enhance the growth of Enterococci, Bile Esculin Agar can be supplemented with 50ml/l horse serum. Inoculate and incubate the test sample in Todd Hewitt Broth. After 24 hours of incubation add two drops of the culture onto the surface of slant or plate media.

### **PRINCIPLE**

Peptone and meat extract serves as sources of carbon, nitrogen, amino acids, vitamins and essential growth nutrients. Bile inhibits most of the other accompanying bacteria.

### INSTRUCTION FOR USE

- Dissolve 31.75 grams in 500 ml purified / distilled water.
- Heat to boiling to dissolve the medium completely. Cool to 45-50°C.
- Add rehydrated contents of 1 vial of Esculin.
- Mix and dispense into tubes or flasks as desired.
- Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes.
- Allow the tubed medium to solidify in slanted position.

### Product Specifications

Ingredients	Gms / Ltr
Peptone	5.000
Meat extract	3.000
Bile	40.000
Ferric citrate	0.500
Agar	15.000

### QUALITY CONTROL SPECIFICATIONS

Appearance of Dehydrated powder: Cream to yellow, homogeneous free flowing powder  
 Appearance of Prepared medium: Amber coloured, clear to slightly opalescent gel forms in Petri plates.

pH (at 25°C) : 6.6± 0.2

### 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.

### PRINCIPLE

The medium contains peptone and beef extract that serve as source of carbon, nitrogen and essential growth factors. Bile Salts do not inhibit enterococci while other Gram-positive bacteria are inhibited. Organisms that hydrolyze esculin produce esculetin which reacts with ferric citrate to form a dark brown or black complex. Ferric citrate acts as an indicator of esculin hydrolysis. Bacteriological agar is the solidifying agent.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Esculin hydrolysis	Incubation Temperature	Incubation Period
Enterococcus faecalis	29212	50-100	Luxuriant	>=70%	Positive reaction, blackening of medium around the colony	35-37°C	24-48 Hours
Proteus mirabilis	25933	50-100	None- Poor	0-10%	Negative reaction	35-37°C	24-48 Hours
Streptococcus pyogenes	19615	50-100	None- Poor	0-10%	Negative reaction	35-37°C	24-48 Hours

**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.

**This product is for research use only.**