# Bile Esculin Agar Base

**M340**

Bile Esculin Agar Base is a differential medium recommended for isolation and presumptive identification of group D Streptococci from food and pharmaceutical products.

## Composition**

| Ingredients | Gms / Litre |
|---|---|
| Peptone | 5.000 |
| Meat extract B # | 3.000 |
| Bile | 40.000 |
| Ferric citrate | 0.500 |
| Agar | 15.000 |
| Final pH ( at 25°C) | 6.6±0.2 |

**Formula adjusted, standardized to suit performance parameters

# Equivalent to Beef extract

## Directions

Suspend 31.75 grams in 500 ml distilled water. Heat to boiling to dissolve the medium completely. Cool to 45-50°C.Add rehydrated contents of 1 vial of Esculin (FD050). Mix and dispense into tubes or flasks as desired. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes. Allow the tubed medium to solidify in slanted position.

## Principle And Interpretation

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 (1). The unique ability of Enterococci to split esculin was reported by Meyer and Schonfeld (2). Enterococci and Group D Streptococci hydrolyse esculin to esculetin and dextrose, which reacts with ferric citrate producing brownish black precipitate (3). The use of esculin hydrolysis in identification of Enterococci was first cited by Rochaix (4). Bile Esculin Agar was originally formulated by Swan (6) for the isolation and identification of Group D Streptococci from food. Facklam and Moody (7, 8) 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 (9) on the basis of esculin hydrolysis. However, other tests such as salt tolerance should be performed for identifying Enterococci (5).

Bile Esculin Agar Base with added supplements is recommended for selective isolation and presumptive identification of group D streptococci from food and pharmaceutical products.

Peptone and meat extract B serves as sources of carbon, nitrogen, amino acids, vitamins and essential growth nutrients. Bile inhibits most of the other accompyning bacteria. 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 (10). To enhance the growth of Enterococci, Bile Esculin Agar can be supplemented with 50ml/l horse serum (3).
Inoculate and incubate the test sample in Todd Hewitt Broth (M313). After 24 hours incubation add two drops of the culture onto the surface of slant or plate media (3, 5).

## Quality Control

**Appearance**
Cream to brownish yellow homogeneous free flowing powder

**Gelling**
Firm, comparable with 1.5% Agar gel

**Colour and Clarity of prepared medium**
Amber coloured, clear to slightly opalescent solution with a bluish tinge forms in Petri plates or in tubes as slants.

**Reaction**
Reaction of 6.35% w/v aqueous solution at 25°C. pH : 6.6±0.2

**pH**
6.40-6.80

**Cultural Response**
Cultural characteristics observed with added Esculin (FD050) in an increased atmosphere of Carbon dioxide, after an incubation at 35-37°C for 18-24 hours .

**Cultural Response**

| Organism | Inoculum (CFU) | Growth | Recovery | Esculin Hydrolysis |
|---|---|---|---|---|
| **Cultural Response** | | | | |
| *Enterococcus faecalis ATCC 29212* | 50-100 | luxuriant | >=50% | positive reaction, blackening of medium around the colony |
| *Proteus mirabilis ATCC 25933* | 50-100 | luxuriant | >=50% | negative reaction |
| *Streptococcus pyogenes ATCC 19615* | 50-100 | none-poor | <=10% | negative reaction |

## Storage and Shelf Life
Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry period on the label.

## Reference
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