

Bile Esculin Agar

Recommended for isolation and presumptive identification of group D Streptococci from food and pharmaceutical products.

REF: BS.1/NZ01.100.0100 100 Gram
 REF: BS.1/NZ01.500.0500 500 Gram

REF: BS.1/NZ01.250.0250 250 Gram

PRINCIPLE AND INTERPERTATION :

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 (8). The unique ability of Enterococci to split esculin was reported by Meyer and Schonfeld (10). Enterococci and Group D Streptococci hydrolyse esculin to esculetin and dextrose, which reacts with ferric citrate producing brownish black precipitate (9). The use of esculin hydrolysis in identification of Enterococci was first cited by Rochaix (12). Bile Esculin Agar was originally formulated by Swan (4) for the isolation and identification of Group D Streptococci from food. Facklam and Moody (2,5) 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 (11) on the basis of esculin hydrolysis. However, other tests such as salt tolerance should be performed for identifying Enterococci (3). The medium is highly nutritious. Peptone and HM peptone B serves as sources of carbon, nitrogen, amino acids, vitamins and essential growth nutrients. Bile 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. 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. Viridians Streptococci sometimes exhibit a weak positive reaction. Also, Leuconostoc, Pediococcus, Lactococcus species causing human infections give a positive bile esculin test (6). To enhance the growth of Enterococci, Bile Esculin Agar can be supplemented with 50ml/L horse serum (9). 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, 9).

MEDIA COMPOSITION

Item	Formula per liter of medium
Peptone	5.00 gm
Beef extract	3.00 gm
Bile salt #	40.0 gm
Esculin	1.00 gm
Ferric citrate	0.5 gm
Agar	15.00 gm

Equivalent to oxgall
 Final pH (at 25oC) 6.6 ± 0.2

PRECAUTIONS AND WARNINGS

Media to be handled by entitled and professionally educated person. Do not ingest or inhale.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
- **S56:** dispose of this material and its container at hazardous or special waste collection point.
- **S57:** use appropriate container to avoid environmental contamination.
- **S61:** avoid release in environment.

For further information, refer to the Bile Esculin Agar material safety data sheet.

STORAGE AND STABILITY

BioScien Bile Esculin Agar Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

PREPARATION

Suspend 64.5 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Mix and dispense into tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the tubed medium to solidify in slanted position.

Deterioration

The color of BioScien Bile Esculin Agar Light yellow to brownish yellow homogeneous free flowing powder.

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile petri dishes
- Incubator
- Autoclave

PERFORMANCE CHARACTERISTICS

Cultural characteristics observed in an increased atmosphere of Carbon dioxide after an incubation at 35-37°C for 18-24

hours.

Organisms (ATCC)	Growth	Esculin hydrolysis
<i>Streptococcus pyogenes</i> ATCC 19615	None-poor	Negative reaction
<i>Enterococcus faecalis</i> ATCC 29212	luxuriant	Positive reaction, blackening of medium around the colony
<i>Proteus mirabilis</i> ATCC 25933	luxuriant	Negative reaction

QUALITY CONTROL

To ensure adequate quality control, it is recommended that positive and negative control included in each run. If control still out of range please contact **BioScien** technical support.

REFERENCE

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

Washington D.C

2. Edberg S. C., Pittman S., and Singer J. M., 1977, J. Clin. Microbiol., 6:111.

3. Facklam R., 1973, Appl. Microbiol., 26:138. Swan, 1954, J. Clin. Pathol., 7:160.

4. Facklam R., 1972, Appl. Microbiol., 23:1131.

5. Facklam R. R and Moody M. D., 1970, Appl. Microbiol., 20(2):245.

6. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.

7. 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

8. Koneman E. W., Allen S. D., Janda W. M., Schreckenberger P. C., Winn W. C. Jr., 1992, Colour Atlas and Textbook of

Diagnostic Microbiology, 4 th Ed., J. B. Lippincott Company

9. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams

and Wilkins, Baltimore.

10. Meyer and Schonfeld, 1926, Zentralbl. Bakteriologie, Parasitenk. Infektionskr. Hyg. Abt. Orig. 99:402

11. Murray P. R., Baron E. J., Jorgensen J. H., Pfaller M. A., Tenover F. C., Tenover F. C., 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.

12. Rochaix, 1924, Compt. Rend. Soc. Biol., 90:771.

13. 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

14. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed.,

APHA Inc., Washington, D.C.

SYMBOLS IN PRODUCT LABELLING

	For in-vitro diagnostic use		Number of <n> test in the pack
	Batch Code/Lot number		Caution
	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		