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Esculin Azide Broth

Designed for selective cultivation and identification of Streptococci.

REF: BS.1/DB01.100.0100	100 Gram	REF: BS.1/DB01.250.0250	250 Gram
REF: BS.1/DB01.500.0500	500 Gram		

CLINICAL SIGNIFICANCE

Esculin Azide Broth is prepared according to a modification of the original formulation described by Isenberg (3). Enterococci possess the ability to hydrolyze esculin, whereas other streptococci generally lack this capability. Swan (7) employed an esculin-containing medium supplemented with 40% bile salts and reported that a positive reaction in Bile Esculin Medium correlated with a serological group D precipitin reaction. Subsequent investigations by Facklam and Moody demonstrated that the bile esculin test is a reliable method for the presumptive identification of group D Streptococci and their differentiation from non-group D Streptococci (2). The present formulation represents a modification of Bile Esculin Agar developed by Isenberg et al., which contained 40 g/L of bile. In Esculin Azide Broth, the bile concentration was reduced to 10 g/L, with the addition of sodium azide to enhance the selective properties of the medium (3). The selectivity of the broth is attributed to the combined action of bile salts and sodium azide, while its nutrient components support the rapid growth of Streptococci. Peptone and yeast extract provide essential nitrogenous nutrients required for bacterial growth. Bile salts primarily inhibit susceptible Gram-positive bacteria, whereas sodium azide suppresses the growth of Gram-negative bacteria. Organisms capable of hydrolyzing esculin convert it into esculetin and dextrose. The liberated esculetin reacts with ferric ammonium citrate to produce a dark brown to black complex, resulting in a characteristic dark brown coloration of the broth.

MEDIA COMPOSITION

Item	Formula per liter of medium g/l
- Peptone	20
- Yeast extract	5
- Bile salt	10
- Sodium citrate	1.0
- Sodium Azide	0.25
- Esculin	1.0
- Ferric ammonium citrate	0.5

Final pH 7.2 ± 0.2 at 25°C

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 Esculin Azide Broth material safety data sheet.

STORAGE AND STABILITY

BioScien Esculin Azide Broth dehydrated media are stable until expiration date stated on label when properly stored 10-30°C. The prepared medium should be stored at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to avoid lump development due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in a 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.

MEDIA PREPARATION

Suspend 37.75 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Dispense in tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Deterioration

The color of ***BioScien*** Esculin Azide Broth is Cream to yellow homogeneous free flowing powder. Colour and Clarity of prepared mediuml is Amber coloured, clear solution having slight purplish tinge. If there are any physical changes, discard the medium. The hydrated medium is Amber coloured clear solution without any precipitate, media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), and contaminations.

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile cups
- Sterile tubes
- Sterile loops
- Incubator

PERFORMANCE CHARACTERISTICS

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature

Cultural characteristics observed after an incubation at 30-35°C for 18-24 hours

Test Organisms	Growth	Esculin hydrolysis
Escherichia coli ATCC 25922	Inhibited	
Enterococcus faecalis ATCC 29212	Luxuriant	positive reaction, blackening of medium
Streptococcus bovis ATCC 27960	Luxuriant	positive reaction, blackening of medium
Streptococcus pyogenes ATCC 19615	Poor growth	negative reaction, no change
Proteus mirabilis ATCC 25933	Poor to fair	negative reaction, no change

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

QUALITY CONTROL

To ensure adequate quality control, it is recommended that positive and negative control included in each run. If control values are found outside the defined range, check the system performance. If control still out of range please contact ***BioScien*** technical support.

REFERENCES

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

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

3. Isenberg, 1970, Clin. Lab. Forum.2. Swan A., 1954, J. Clin. Pathol., 7:1603. Facklam R. R. and Moody M. D., 1970, Appl. Microbiol., 20:2451. Isenberg, 1970, Clin. Lab. Forum.

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

5. 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.

6. 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.

7. Swan A., 1954, J. Clin. Pathol., 7:160 8. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.