

BILE ESCULIN AZIDE AGAR

BEAA-OEP-500

- **Principle**

Bile Esculin Azide Agar is a selective medium used for the isolation and presumptive identification of faecal streptococci (enterococci). The medium contains tryptone, meat peptone, yeast extract, oxgall, sodium chloride, esculin, ferric ammonium citrate, sodium azide and agar. Tryptone and meat peptone provide nitrogen, amino acids and other essential nutrients required for bacterial growth, while yeast extract supplies additional growth factors, including vitamins. Oxgall (bile salts) acts as a selective agent by inhibiting Gram-positive bacteria other than enterococci. Sodium chloride helps to maintain osmotic balance. Esculin, a glycoside, serves as a carbon source and as the substrate for the differential reaction. Ferric ammonium citrate functions as an indicator of esculin hydrolysis.

Microorganisms capable of hydrolysing esculin produce aesculetin and dextrose. Aesculetin reacts with ferric ions to form a dark brown to black complex, resulting in visible blackening of the medium. Sodium azide further enhances selectivity by inhibiting most accompanying Gram-negative bacteria. Agar is included as the solidifying agent.

The medium is dispensed in tubes as slants. A positive reaction is indicated by blackening of more than half of the slant within 24 - 48 hours. If blackening is completely absent, or if less than half of the slant is blackened within 24 - 48 hours, the test is considered negative.

The medium may be supplemented with 50 mL/L horse serum to enhance the growth of enterococci.

- **Regulatory compliance**

This product is manufactured under a quality management system in accordance with ISO 9001 and ISO 13485, and its formulation and quality control comply with applicable international standards, such as ISO 11133, where relevant.

For this specific medium, compliance is also established with the relevant requirements of ISO 7899-1:1984.

- **Composition**

Ingredients	g/L
Tryptone	17.00
Meat peptone	3.00
Yeast extract	5.00
Oxgall	10.00
Sodium chloride	5.00
Ferric ammonium citrate	0.50
Esculin	1.00
Sodium azide	0.15
Agar	15.00

- **Preparation**

Dissolve 56.70 grams in 1000 ml distilled water. Boil to dissolve the medium completely. Sterilize by autoclaving at 121°C for 15 min (Overheating may causes darkening of medium), cool it to 42-45°C and dispense in desired (Test tubes to form butt and in petri plates for isolation and identification). Ensure complete solidification and inoculate test sample aseptically.

- **Applications and use**

Recommended for isolation and presumptive identification of faecal Streptococci.

- **Quality control**

Solubility	w/o rests
Appearance	Fine powder
Colour of the dehydrated medium	Beige
Colour of the prepared medium	Amber
Final pH (25 °C)	7.2 ± 0.2

- **Microbiological test**

Microorganism	ATCC	Growth	Recovery	Esculin hydrolysis
<i>Enterococcus faecalis</i>	14506	Luxuriant	≥ 60%	Positive (Black colour)
<i>Staphylococcus aureus</i>	25923	Good	≥ 40%	Negative
<i>Proteus mirabilis</i>	12453	Good	≥ 40%	Negative
<i>Escherichia coli</i>	8739	Inhibited	-	-

- **Storage**

The product is highly hygroscopic; keep the container always closed and store it properly as per the conditions mentioned on the label. The declared expiry is valid only when stored as per the conditions mentioned on the label. Temp. Min.:2 °C Temp. Max.:25 °C.

Note: Sterilize media immediately after reconstitution.

- **Bibliography**

Atlas, R. M. (2005). Handbook of media for environmental microbiology. CRC press.

Difco Manual (1998). 11th Edition. Difco Laboratories., Division of Becton Dickinson and Company, Sparks, Maryland, USA.

Facklam, R. R., and M. D. Moody. (1970). Presumptive identification of group D streptococci: The bile-esculin test. Appl. Microbiol. 20:245.

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

Swan, A. (1954). The use of bile-esculin medium and of Maxted's technique of Lancefield grouping in the identification of enterococci (group D streptococci). J. Clin. Pathol. 7:160.

- **Product use limitation**

This product is developed, designed and supplied exclusively for research use only. It is not intended for diagnostic applications or drug development, and it is not suitable for administration to humans or animals.