

## BILE ESCULIN AGAR ISO 10273

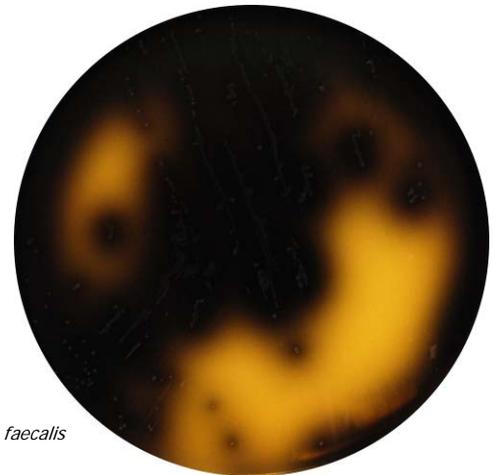
**CAT N°: 1031**

For the isolation and presumptive identification of enterococci and for studies of fermentation of esculin by *Yersinia* according to ISO 10273

### FORMULA IN g/l

Bile Salts	40.00	Esculin	1.00
Meat Peptone	5.00	Ferric Citrate	0.50
Meat Extract	3.00	Bacteriological Agar	15.00

**Final pH 6.6 ± 0.2 at 25°C**



*Enterococcus faecalis*  
ATCC 11700

### PREPARATION

Suspend 64.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. Overheating can cause darkening of the medium. If tubes are used, allow cooling in a slanted position. The prepared medium should be stored at 8-15°C. The color is tournasol.

The dehydrated medium should be homogeneous, free-flowing and toasted in color. If there are any physical changes, discard the medium.

### USES

BILE ESCULIN AGAR is ideal for the isolation and differentiation of intestinal enterococci, based on Esculin hydrolysis in the presence of bile.

Organisms positive for Esculin hydrolysis hydrolyze the glycoside esculin to esculetin and dextrose. The esculetin reacts with the Ferric citrate to form a dark brown or black colony. Bile Salts do not inhibit enterococci while other Gram positive bacteria are inhibited. Beef extract and Peptone supply the nutrients essential for growth. Bacteriological agar is the solidifying agent.

Tolerance to bile and the ability to hydrolyze esculin constitutes a reliable presumptive test for the identification of Enterococci. The brown color (positive reaction) around the colonies appears after 18 - 24 hours of incubation at a temperature of 35 ± 2°C. Positive cultures are confirmed on KAA Confirmatory Agar (Cat. 1027) or KF Streptococcal Agar (Cat. 1034).

The presence of intestinal enterococci, is an indicator for faecal contamination, especially when the contamination occurred a long before and the less resistant coliform bacteria, including *Escherichia coli*, may already be dead when the analysis is carried out.

ISO 10273 recommends this medium for fermentation studies of esculin by *Yersinia*. An esculin test shall be carried out to determine presumed pathogenicity since pathogenic *Yersinia enterocolitica* strains are esculin negative. Streak the slant surface of the agar. Inoculate 30°C for 24 hours. A black halo around the colonies indicates a positive reaction. This test for fermentation of esculin is equivalent to the test for fermentation of salicin.

### MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	Esculin Hydrolysis
<i>Enterococcus faecalis</i> ATCC 11700	Good	+
<i>Enterococcus faecalis</i> ATCC 19433	Good	+
<i>Enterococcus faecium</i> ATCC 8043	Good	+
<i>Streptococcus pyogenes</i> ATCC 12344	Null	-
<i>Streptococcus pneumoniae</i> ATCC 6301	Null	-
<i>Staphylococcus aureus</i> ATCC 25923	Good	+ light
<i>Escherichia coli</i> ATCC 25922	Light	-
* <i>Yersinia enterocolitica</i> ATCC 27729	Good	-

\*ISO 10273 Microbiology of food and animal feeding stuffs- Horizontal method for the detection of presumptive pathogenic. *Yersinia enterocolitica*

\*Inoculate at 30°C for 24 hours

## BIBLIOGRAPHY

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

Farmer J.J. III 1995 Enterobacteriaceae P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover and R.H. Tenover (eds) Manual of clinical microbiology, 6<sup>th</sup> ed. American Society for Microbiology, Washington, D.C.



## STORAGE

Once opened keep powdered medium closed to avoid hydration.



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