



Streptococcus Agalactiae Selective Agar Base

M1257

Streptococcus Agalactiae Selective Agar is recommended for selective isolation of *Streptococcus agalactiae* from dairy products.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	10.000
Meat extract	5.000
Sodium chloride	5.000
Esculin	1.000
Thallos sulphate	0.333
Crystal violet	0.0013
Agar	13.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 34.34 grams in 940 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C and add 60 ml defibrinated blood and 25ml Staphylococcus β-toxin. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Streptococcus agalactiae is a gram-positive *Streptococcus* characterized by the presence of group B Lancefield antigen. *S. agalactiae* exhibits beta haemolytic reaction. On Blood agar plate, it forms zones of haemolysis that are slightly bigger than the size of colonies formed. Group B streptococci hydrolyze sodium hippurate and give a positive response in the CAMP test. *S. agalactiae* is also sensitive to bile and will lyse in its presence. Streptococcus Agalactiae Selective Agar was formulated by Hauge and Kohler-Ellingsen (1) for the isolation of *S. agalactiae*, the causative agent of mastitis in cattle.

Differentiation between *Streptococcus* species is done on the basis of esculin hydrolysis seen as dark brown colour due to formation of an esculin-thallium complex. Thallos sulphate and crystal violet inhibit the accompanying bacterial flora. *Staphylococcus* β-toxin attacks the erythrocytes present in the medium in such a way that they may be completely haemolyzed.

S. agalactiae is not haemolytic on simple blood agar. Thus *S. agalactiae* can be distinguished from obligate, non-haemolyzing colonies.

S. agalactiae forms dove-blue coloured smooth colonies surrounded by zones of haemolysis. Further identification is done by using biochemical and serological methods, but primarily by using CAMP test (2).

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.3% Agar gel.

Colour and Clarity of prepared medium

Basal medium forms light purple coloured, clear to slightly opalescent gel. On addition of blood, red coloured opalescent gel forms in Petri plates

Reaction

Reaction of 3.43% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

M1257: Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Blue colony	Haemolysis
Cultural Response					
<i>Escherichia coli</i> ATCC 25922	$\geq 10^3$	inhibited	0%		
<i>Enterococcus faecalis</i> ATCC 29212	50-100	good-luxuriant	$\geq 50\%$	variable reaction	alpha
<i>Pseudomonas aeruginosa</i> ATCC 27853	$\geq 10^3$	inhibited	0%		
<i>Staphylococcus aureus</i> ATCC 25923	$\geq 10^3$	inhibited	0%		
<i>Streptococcus agalactiae</i> ATCC 13813	50-100	luxuriant	$\geq 50\%$	positive	beta
<i>Streptococcus agalactiae</i> ATCC 27956	50-100	luxuriant	$\geq 50\%$	positive	beta
<i>Streptococcus cremoris</i> ATCC 19257	50-100	luxuriant	$\geq 50\%$	variable reaction	alpha
<i>Streptococcus pneumoniae</i> ATCC 6301	50-100	luxuriant	$\geq 50\%$	negative	alpha
<i>Streptococcus pyogenes</i> ATCC 19615	50-100	luxuriant	$\geq 50\%$	negative	beta
<i>Streptococcus lactis</i> ATCC 19435	$\geq 10^3$	inhibited	0%		

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

1. Hauge S. T. and u Kohler-Ellingsen J., 1953, Nord. Vet. Med., 5:539.
2. Christie R., Atkins N. E. and Munch-Petersen E., 1944, Aust. J. Exp. Biol. Med. Sci., 22:197.

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