

## TM 2345 - STREPTOCOCCUS AGALACTIAE SELECTIVE AGAR BASE

### INTENDED USE

For selective isolation of *Streptococcus agalactiae* from dairy products.

### SUMMARY AND EXPLANATION

*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 for the isolation of *S. agalactiae*, the causative agent of mastitis in cattle.

### COMPOSITION

Ingredients	Gms / Ltr
Peptic digest of animal tissues	10.000
Meat extract	5.000
Esculin	1.000
Agar	13.000
Thallous sulphate	0.333
Crystal Violet	0.0013
Sodium chloride	5.000

### PRINCIPLE

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. Thallous sulphate and crystal violet inhibit the accompanying bacterial flora. Staphylococcus  $\beta$ -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.

### INSTRUCTION FOR USE

- Dissolve 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  $\beta$ -toxin.
- Mix well and pour into sterile Petri plates.

### QUALITY CONTROL SPECIFICATIONS

**Appearance of Powder** : Cream to yellow homogeneous free flowing powder.  
**Appearance 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.  
**pH (at 25°C)** : 7.4  $\pm$  0.2

### INTERPRETATION

Cultural characteristics observed with added 60 ml defibrinated blood, after incubation.



Microorganism	ATCC	Inoculum (CFU)	Growth	Recovery	Blue colony	Hemolysis	Incubation Temperature	Incubation Period
<i>Streptococcus agalactiae</i>	13813	50-100	Luxuriant	>=70%	Positive	Beta	35-37°C	24-48 Hours
<i>Streptococcus pneumoniae</i>	6301	50-100	Luxuriant	>=70%	Negative	Alpha	35-37°C	24-48 Hours
<i>Streptococcus cremoris</i>	19257	50-100	Luxuriant	>=70%	Variable reaction	Alpha	35-37°C	24-48 Hours
<i>Streptococcus agalactiae</i>	27956	50-100	Luxuriant	>=70%	Positive	Beta	35-37°C	24-48 Hours
<i>Streptococcus pyogenes</i>	19615	50-100	Luxuriant	>=70%	Negative	Beta	35-37°C	24-48 Hours
<i>Escherichia coli</i>	25922	>=10 <sup>3</sup>	Inhibited	0%	-	-	35-37°C	24-48 Hours
<i>Enterococcus faecalis</i>	25912	50-100	Good-luxuriant	>=50%	Variable	Alpha	35-37°C	24-48 Hours

#### PACKAGING:

In pack size of 500 gm bottles.

#### STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers below 25°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

**Product Deterioration:** Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

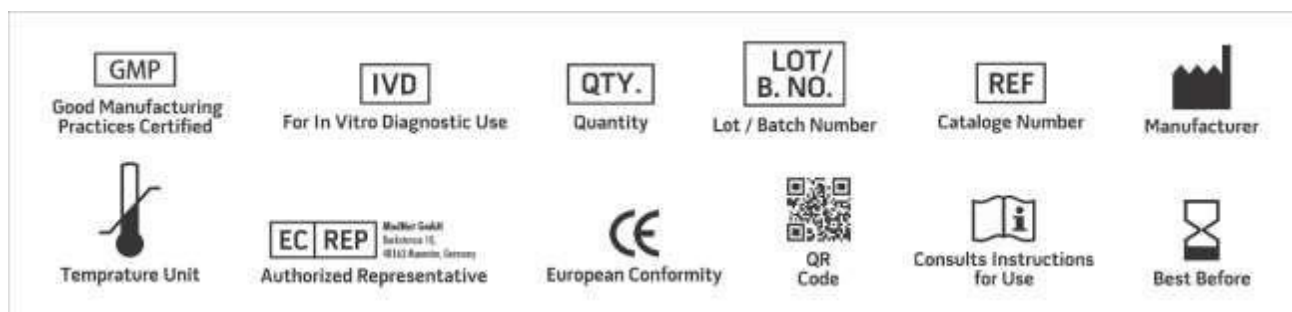
#### DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

#### REFERENCES

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.





**NOTE:** Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

**\*For Lab Use Only**  
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