PRODUCT SPECIFICATION SHEET

Sheep Blood Agar Plate (RP022)

Intended Use

Sheep Blood Agar Plate (RP022) medium with 5-7% defibrinated sheep blood, is recommended for cultivation of fastidious organisms and studying haemolytic reactions.

Product Summary and Explanation

Haemolysins are exotoxins produced by bacteria that lyse red blood cells. The haemolytic reaction can be visualized on blood agar plates. On blood agar plates colonies of haemolytic bacteria may be surrounded by clear, colourless zone where the red blood cells have been lysed and the haemoglobin destroyed to a colourless compound. This is beta haemolysis. Other types of bacteria can reduce haemoglobin to methaemoglobin which produces a greenish zone around the colonies and is called alpha haemolysis.[1] Gamma haemolysis is no haemolysis where no change in the medium is observed.[2] The Sheep Blood Agar was developed to meet the demand for an especially nutritious blood agar base which would permit the maximum recovery of organisms without interfering with their haemolytic reactions when used with sheep blood. Sheep Blood Agar Base is based on the formulation of Blood Agar Base No. 2. Blood Agar Base No. 2 when supplemented with sheep blood was occasionally found to result in a mixed haemolytic reaction (alpha and beta haemolysis) for some Group A Streptococci (*Streptococcus pyogenes*). These mixed haemolytic reactions were due to trace amounts of fermentable carbohydrates in yeast extract and the physiological differences of sheep blood when compared to horse blood.[3]

Having identified the causes of the mixed haemolytic reactions, the Sheep Blood Agar Base was formulated to be compatible with sheep blood. Comparisons with other blood agar bases supplemented with sheep blood have shown that with Sheep Blood Agar Base the growth of many bacteria especially the fastidious streptococci is considerably improved, and the expected beta haemolytic reaction is achieved with *Streptococcus pyogenes*.

Principles of the Procedure

Sheep Blood Agar Plate contains casein enzymic hydrolysate and yeast extract provide nitrogen, carbon, amino acids and vitamins. Peptic digest of animal tissue is the nitrogen source. Sodium chloride maintains the osmotic balance. Sheep Blood Agar Base showed considerable improvement and the expected beta haemolytic reactions with *S. pyogenes* in comparison to other blood agar bases supplemented with blood.

Formula / Liter

Ingredients	Gms / Litre
Casein enzymic hydrolysate	14.00
Peptic digest of animal tissue	4.50
Yeast extract	4.50
Sodium chloride	5.00
Agar	12.50
Sheep Blood	5.00
Final pH: 7.3 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

1. Prepared plated media are For *in vitro* Diagnostic Use or For Laboratory Use as labeled.
2. Directions for use should be read and followed carefully.
3. If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.
4. Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures, since it must be assumed that all specimens/samples collected might contain infectious microorganisms.

Product Deterioration

Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

Directions
Either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically on the plate.

Quality Control Specifications

Appearance	Sterile Blood Agar in 90 mm disposable plates
Colour	Red coloured medium
Reaction	7.10- 7.50
Quantity of medium	25ml of medium in disposable plate

Sterility Check: Passes release criteria.

Expected Cultural Response: Cultural characteristics observed after incubation at 35-37°C for 18-48 hours.

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Growth	Recovery	Haemolysis
1.	*Streptococcus pyogenes ATCC 19615*	50-100	good-luxuriant	>=70%	beta
2.	*Staphylococcus aureus ATCC 6538*	50-100	good-luxuriant	>=70%	beta
3.	*Enterococcus faecalis ATCC 29212*	50-100	good-luxuriant	>=70%	beta
4.	*Escherichia coli ATCC 25922*	50-100	good-luxuriant	>=70%	none

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure
Refer appropriate references for standard test procedures.

Results
Refer appropriate references and procedures for interpretation of results.

Storage
On receipt, store plates at 2-8°C. Freezing and overheating must be avoided. Allow the medium to warm to room temperature before inoculation. Media containing dyes should be protected from light.

Expiration
Refer to the expiration date stamped on the pack. Prepared plates stored in their original sleeve wrapping at 2-8°C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times, including up to 6 weeks for mycology media and up to 8 weeks for mycobacteriology media.

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

Limitations of the Procedure
1. Some diagnostic tests may be performed with the primary plate. However, a pure culture is recommended for the majority of biochemical tests and other identification procedures.
2. Consult appropriate references for further information.

Packaging
Product Name : Sheep Blood Agar Plate
Product Code : RP022
Available Pack sizes : Pack of 10 plates

References

1. Pelczar M. J. Jr., Reid R. D., Chan E. C. S., 1977, Microbiology, 4th Ed., Tata McGraw-Hill Publishing Company Ltd, New Delhi.
2. Koneman E. W., Allen S. D., Janda W. M., Schreckenberger P. C., Winn W. C. Jr., 1992, Colour Atlas and Textbook of Diagnostic Microbiology, 4th Ed., J. B. Lippinccott Company.
3. Spector W. S., (Ed.), 1961, Handbook of Biological Data, W. B. Saunder Company, Philadelphia and London.

Further Information

For further information please contact your local MICROMASTER Representative.

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