PRODUCT INFORMATION

Product Type: PETRI DISHES 90mm

Cat No. PD005 - BLOOD AGAR BASE + DEF. SHEEP BLOOD

Intended Use:
In vitro diagnostic. Non selective, general-purpose medium supplemented with defibrinated animal blood, for the isolation and cultivation of fastidious and non-fastidious microorganisms from clinical specimens and other materials and for the determination of their haemolytic properties.

Principle and Uses:

Blood Agar Base is a general-purpose medium, Beef extract and tryptose are a source of carbon, nitrogen and trace elements are necessary for microbial growth; sodium chloride contributes to the osmotic balance of the medium. the medium is intended for the isolation and cultivation of fastidious and non-fastidious microorganisms from clinical specimens and other materials, and for the determination of haemolytic properties of *streptococci*, *staphylococci* and other microorganisms. With the addition of sheep blood, the medium is particularly suitable for the isolation of *Streptococcus pyogenes.*

Test Procedure
Allow plates to come to room temperature and to dry the surface of the medium. After Inoculation Incubate at 35-37°C in aerobic conditions with or without 5-10% CO2, and record the results after 18-24, 48 and, if necessary, 72 hours. The user is responsible for choosing the appropriate incubation time, temperature and atmosphere depending on the processed specimen, the requirements of organisms to be recovered and the local applicable protocols.

Reading And Interpretation
After incubation, observe the bacterial growth and record the specific morphological, chromatic, haemolytic characteristics of the colonies.
By cultivation on PD005 plates bacteria can be differentiated based on their capacity to secrete haemolysins. The haemolysis will cause a clearing zone of the blood agar around the colonies. Bacteria can cause different types of haemolysis:

1. **α-haemolysis**: partial haemolysis of the red blood cells to produce a greenish-grey or brownish discoloration around the colonies.
2. **β-haemolysis**: complete haemolysis of red blood cells resulting in a clear zone around the colonies
3. **γ or non-haemolysis**: no haemolysis of red blood cells, no change of the medium under and surrounding the colonies.
4. **α-prime haemolysis**: a small zone of complete haemolysis that is surrounded by an area of partial lysis with green discoloration; this type of haemolysis is uncommon.

Below summary of colonies characteristics of some microorganisms that can be isolated on blood agar sheep plates:

- The colonies of *Group A streptococci* are surrounded by a well-defined zone of complete haemolysis, usually two or three times the diameter of the colony.
- The colonies of *group B streptococci* are surrounded by a much smaller zone of complete haemolysis and some strains do not lyse the blood at all.
- The appearance of surface or sub-surface β-haemolytic *group C* and *group G streptococcal* colonies do not differ sufficiently from that of group A colonies to be of any value in identification.
- *Group D streptococcal* colonies are non-haemolytic.
- Pneumococcal colonies, when the culture has been incubated in CO2 incubators, are surrounded by a fairly large zone of α-haemolysis.
- The viridans streptococcal colonies may be surrounded by a small zone of α-haemolysis or have no zone of haemolysis; rarely they show an α-prime haemolysis.
- *Staphylococci* colonies are yellow or white with or without the β-haemolysis zone.
- *Listeria monocytogenes* colonies are surrounded by a small β-haemolytic zone.

Once colonies have grown on blood agar plates, user must differentiate potential pathogens requiring identification and antimicrobial testing from contaminants that represent members of normal microbiota.

Limitations Of the Method

- Depending on the specimens analyzed and the microorganisms being tested for, it is recommended for the examination of clinical specimens to use also additional media such us selective media and Chocolate Agar.
- The growth and type of haemolysis depend on the metabolic requirements of organisms; it is possible that some strains do not grow and/or can demonstrate haemolytic patterns other than expected.
- *Haemophilus influenzae*, which requires both factor X and factor V, will not grow on this medium supplemented with sheep blood; *Neisseria, Mycobacterium, Bordetella* and other microorganisms with highly specific nutritional requirements do not grow adequately; for the detection of these organisms, specific culture media should be used.
- The hemolytic reactions of some strains of *group D streptococci* are influenced by the type of blood used: they are beta-hemolytic with horse, human and rabbit blood and alpha-haemolytic with sheep blood.
- The incubation atmosphere influences the haemolytic reactions of beta-haemolytic *streptococci:* for optimal performance, incubate the plates in aerobic conditions with 5-10% CO_2 or in anaerobic conditions.

Composition

Beef extract - 10 g/L
Tryptose - 10 g/L
Sodium chloride - 5 g/L
Agar - 15 g/L
Donor Sheep Blood - 50ml/L

Storage: 2-8 °C
Appearance: Cherry red opaque
pH Range: 7.2 - 7.6

Package contents: 10 plates in a package
Exp. Date: Printed on label and on the item.
Required materials not supplied: Laboratory equipment as required.

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Warning and Precautions - For professional use only. Follow good microbiological lab practices while handling specimens and culture. Do not use Petri dishes if they show evidence of microbial contamination, discoloration, drying, cracking, or other signs of deterioration. Avoid freezing and overheating. The Petri Dishes may be used / inoculated up to the expiration date and incubated for the recommended incubation times. After use and prior to discarding, specimen containers and all contaminated material, including the used culture media and contaminated culture containers, must be sterilized or incinerated by validated procedures. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
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. Storage Instructions: On receipt, store plates in the dark at 2–8 °C. Avoid freezing and overheating. Do not open until ready to use.

Waste Disposal
After interpretation all items should be destroyed by standard incineration methods.

Performance Testing Results:
GPT: Inoculum 10-100 cfu

TEST	ATCC	Incubation Temp. (℃)	Incubation Cond	Reaction	
Staphylococcus aureus	25923	33-37 ℃	Aerobic, 24 hours	Growth	Beta hemolytic reaction
Staphylococcus aureus	W.S	33-37 ℃	Aerobic, 24 hours	Growth	Beta hemolytic reaction
Streptococcus pyogenes group A	19615	33-37 ℃	Aerobic, 24 hours	Growth	Beta hemoytic reatcion, sensitive to bacitracin. zone of inhibition >14 mm.
Streptococcus pneumoniae	49619	33-37 ℃	Aerobic, 24 hours	Growth	Alpha hemolytic reaction