



Anaerobic Agar without Dextrose

M230

Anaerobic Agar without Dextrose is used for carbohydrate fermentation studies and for studies of haemolytic activity of Clostridia, Streptococci and other organisms.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	17.500
Sodium chloride	2.500
Sodium thioglycollate	2.000
Sodium formaldehyde sulphonylate	1.000
Methylene blue	0.002
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 38 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Anaerobic Agar without Dextrose is devoid of dextrose (1) and is used for carbohydrate fermentation studies and for studies of haemolytic activity of Clostridia, Streptococci and other organisms. Casein enzymic hydrolysate is the only source of nutrient present. Other peptones, rich in carbohydrates like yeast extract or papaic digest of soyabean meal are not used in this medium as this medium favours distinct haemolytic reactions. Addition of sodium thioglycollate and sodium formaldehyde sulphonylate create conditions necessary for cultivation of anaerobic bacteria which is indicated by methylene blue dye present in the medium. Sodium chloride maintains osmotic equilibrium. For haemolytic tests anaerobic blood agar plates may be prepared in one of the following ways;

Sterile blood in about 0.7 ml amount and small inoculum may be mixed with 25-50 ml of cooled agar and mixture is poured into the Petri plate filling it up to 3/4. After solidification the lid is replaced with Brewer Anaerobic Petri plate cover.

An ordinary sterile Blood Agar plate (made from Blood Agar Base or Tryptone Soya Agar) may be streaked with a culture. Melted and cooled Anaerobic Agar without Dextrose is then poured over the Blood Agar to provide the proper depth. After solidification the lid is replaced with anaerobic Petri plate cover.

The anaerobic cover should not rest on the Petri plate bottom: its inner ridge should seal the agar, and the medium within the ridge should not touch the cover at any point. The medium should be cherry red in colour after addition of blood.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Light amber coloured clear to slightly opalescent gel forms in Petri plates that becomes greenish due to aeration on standing.

Reaction

Reaction of 3.8% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

M230: Cultural characteristics observed under anaerobic condition after an incubation at 35-37°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery
Cultural Response			
<i>Clostridium butyricum</i> ATCC 13732	50-100	good-luxuriant	>=50%
<i>Clostridium perfringens</i> ATCC 12919	50-100	good-luxuriant	>=50 %
<i>Clostridium sporogenes</i> ATCC 11437	50-100	good-luxuriant	>=50%

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. Vera J., 1942, J. Bact., 44:497

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