

## ANAEROBIC AGAR

**CAT Nº: 1000**

For the cultivation of anaerobes, especially *Clostridium*

### FORMULA IN g/l

Casein Peptone	17.50	Sodium Formaldehyde Sulfoxylate	1.00
Dextrose	10.00	L-Cystine	0.40
Soy Peptone	2.50	Methylene Blue	0.002
Sodium Chloride	2.50	Bacteriological Agar	15.00
Sodium Thioglycollate	2.00		

**Final pH 7.2 ± 0.2 at 25°C**

### PREPARATION

Suspend 51 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121 °C for 15 minutes. The prepared medium should be stored at 8-15°C. The color is white with a blue tint. The medium can be incubated in an anaerobic jar or with Brewer lids for anaerobiosis.

The dehydrated medium should be homogeneous, free-flowing and clear beige in color. If there are any physical changes, discard the medium.

### USES

ANAEROBIC AGAR is used for cultivation of anaerobic microorganisms. Anaerobic bacteria are unable to use oxygen as a terminal electron acceptor.

Casein peptone and Soy peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Dextrose is the fermentable carbohydrate providing carbon and energy. Sodium thioglycollate and Sodium formaldehyde sulfoxylate act as reducing agents generating a low oxidation-reduction potential, thus securing good anaerobic conditions. Methylene blue acts as the redox indicator, the blue color indicating the presence of oxygen.

The testing procedures can be carried out using standard Petri dishes or Brewer's Anaerobic Agar Plates, both with the medium cooled to 45 - 50°C.

The seeding of the sample (clinical or food) can be performed by surface inoculation or by pour plate method. Normally the sample should never be heated to destroy the vegetative forms of the aerobe, as the anaerobic non-spore formers will also be destroyed. Nevertheless, sometimes it could be useful to heat the sample when spore formers such as *Clostridium* are sought, except *C. perfringens*, which rarely forms spores. When heating is indicated, warm the sample suspended in a liquid diluent (peptone water, buffering phosphate solution, etc.) to 70 - 80°C for 10 minutes. Incubate at 35 ± 2°C for 18 - 48 hours.

The plates of Anaerobic Agar can also be incubated in a normal atmosphere covering the surface of the plates with a Brewer lid. When growth is observed, open the plate and pick the desired colonies. Incubate longer if necessary. If the medium has not been prepared fresh before use, it is necessary to heat and remelt to expel the dissolved oxygen.

Thioglycollate Medium (Cat. 1508) without Indicator is an excellent enrichment broth and, frequently using it previously, yields better results than direct seeding.

## MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of  $35 \pm 2^\circ\text{C}$  and observed after 18 - 48 hours under anaerobic conditions

Microorganisms	Growth
<i>Clostridium butyricum</i> ATCC 9690	Good
<i>Clostridium perfringens</i> ATCC 12919	Good
<i>Clostridium sporogenes</i> ATCC 11437	Good

## BIBLIOGRAPHY

Standard Methods for the Examination of Water and Wastewater 1 5th Ed. American Public Health Association, Inc, Washington, D.C. 1980. Andrew, W.H.C.D. Diggs, and C.R. Wilson, 1975.

Evaluation of medium for the rapid recovery of *Escherichia coli* from shellfish. App. Microbiol. 29: 130-131



## STORAGE

Once opened keep powdered medium closed to avoid hydration.

