

## TECHNICAL DATA SHEET

### Chocolate Agar Base

#### Principle

Chocolate Agar Base, composed of proteose peptone that provides the organism with nitrogenous nutrients, amino acids, and other elements essential for the growth of the organisms. Dextrose is carbon and energy source. Phosphates buffer the medium. Sodium chloride maintains osmotic balance. Agar is a solidifying agent. The selectivity is provided by addition of glutamine, coenzymes, carboxylase, hematin and growth factors required by fastidious organisms. Thayer-Martin Media is a chocolate agar supplemented with vancomycin, nystatin, and colistin to inhibit the normal flora, including nonpathogenic *Neisseria* for the selective isolation of *N. gonorrhoea* and *N. meningitidis*.

**Use:** For the isolation and cultivation of fastidious microorganisms like *Neisseria gonorrhoeae*.

#### Contents\*

Ingredients	Gram/Litre
Protease Peptone	20.00
Dextrose	0.50
Sodium Chloride	5.00
Disodium hydrogen Phosphate	5.00
Agar	15.00
pH at 25°C	7.2 ±0.2

\* Formula adjusted for optimum performance and parameters

**Directions:** Dissolve 45.50 grams in 495 ml distilled water. Boil to dissolve the medium completely and sterilize by autoclaving at 15 lbs pressure (121 °C) for 15 min, cool it to 42-45 °C. Add 500 ml of sterile hemoglobin solution and mix thoroughly and distribute aseptically in petri plates. Ensure complete solidification and inoculate test sample aseptically. Sterile 2% hemoglobin solution: Add 10 ml of hemoglobin to distilled water and bring volume to 500 ml. Mix thoroughly and autoclave for 15 min at 15lbs pressure at 21°C. Cool at 45°C -50°C. The media can also be enriched with yeast autolysate supplement or vitamin growth supplement reconstituted. Mix well before pouring.

# OXFORD LAB FINE CHEM LLP

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**Regd Office:** Unit no 12, 1st Floor,  
Neminath Industrial Estate No.6,  
Navghar, Vasai (East), Palghar - 410210.  
Maharashtra, INDIA.

**Tel:** +91 250 2390032 / 2390989 / 2390990  
**Email:** sales@oxfordlabchem.com /  
info@oxfordlabchem.com  
**Web:** www.oxfordlabchem.com



## Specimens types analyzed

Pharmaceutical samples, clinical and non-clinical samples etc.

## Precautions to be taken

These microbial media are intended for the in-vitro use only. All the handling, experiments, storage, and discarding should be performed with the help of skilled and knowledgeable technicians and as per the established guidelines. The material should be disposed only after proper sterilization by autoclaving. Please go through the MSDS of the media to avoid any accidents or in emergency.

## Performance and Evaluation

The expected performance of the medium is liable to use as per the direction on the label when stored at optimum conditions and within expiry date.

## Quality Control

<b>Appearance</b>	<b>Beige colored free flowing, homogeneous powder</b>
<b>Reaction of 4.55% solution</b>	<b>7.2 ±0.2 at 25 °C</b>
<b>pH</b>	<b>7.00- 7.40</b>
<b>Gelling</b>	<b>Firm comparable with 1.5 % agar gel</b>
<b>Color and clarity of ready medium</b>	<b>Light amber colored opalescent gel (without hemoglobin solution)</b>
<b>Color and clarity of ready medium</b>	<b>Chocolate brown colored opaque gel (with hemoglobin solution)</b>
<b>Indicative properties</b>	<b>Optimum at ≤ 100 CFU at 32-37 °C for 18-48 h</b>
<b>Growth Promotion properties</b>	<b>Best at ≤ 100 CFU at 32-37 °C for 18-72 h</b>
<b>Negative control</b>	<b>Performed using sterile distilled water</b>

## Different Microbial Response

<b>Organism</b>	<b>ATCC</b>	<b>Inoculum</b>	<b>Growth</b>	<b>Recovery</b>	<b>Incubation</b>
<i>Neisseria gonorrhoeae</i>	19424	50-100	Luxuriant	≥ 70%	33-37 °C, 18-48 h
<i>Streptococcus pneumoniae</i>	6303	50-100	Luxuriant	≥ 70%	33-37 °C, 18-48 h

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**Storage and Shelf Life:** Hygroscopic; keep container tightly closed. Store in cool dry place.

**Disposal:** To avoid the contamination or propagation of any hazardous microbes the used, unusable or modified preparation of this product must be disposed after autoclaving after completion of task.

## Reference

1. Atlas, R. M. (2005). Handbook of media for environmental microbiology. CRC press.
2. Difco Manual (1998). 11th Edition. Difco Laboratories., Division of Becton Dickinson and Company, Sparks, Maryland, USA.
3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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