

## TECHNICAL DATA SHEET

### Reinforced Clostridial Broth

#### Principle

Reinforced medium for clostridia is used for enumerating and cultivating *Clostridia*. It is also recommended by USP, IP and EP for microbial limit testing of pharmaceutical products and raw material used in pharmaceutical industries. Media is composed of meat extract (equivalent to beef extract), peptone, yeast extract, soluble starch, glucose monohydrate, cysteine hydrochloride, sodium chloride, sodium acetate and small quantity of agar. Meat extract, peptone, yeast extract, starch, and cysteine hydrochloride provide nutrients and co-factors required for growth of *Clostridia*. In addition to that cysteine hydrochloride acts as reducing agent and soluble starch absorb toxic metabolites from the medium. Glucose monohydrate is included in the medium as an energy source. Sodium chloride maintains osmotic equilibrium. Sodium acetate acts as buffering agent. Less quantity of agar makes the medium semi solid and helps in maintaining anaerobic conditions.

**Use:** For cultivation and enumeration of Clostridia and other anaerobes.

#### Contents\*

Ingredients	Gram/Litre
Meat Extract#	10.00
Peptone	10.00
Yeast Extract	3.00
Soluble Starch	1.00
Glucose Monohydrate	5.00
Cysteine Hydrochloride	0.50
Sodium Chloride	5.00
Sodium Acetate	3.00
Agar	0.50
pH at 25°C	6.8 ±0.2

**Directions:** Dissolve 38.00 grams in 1000 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 and distribute aseptically in test tubes. Allow to cool and inoculate test sample aseptically.

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## Specimens' types analyzed

Pharmaceutical samples, clinical and non-clinical samples etc.

## Precautions to be taken

These plant tissue culture 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

Appearance	Light beige colored free flowing, homogeneous powder
Reaction of 3.8% solution	6.8 ±0.2 at 25 °C
pH	6.60- 7.00
Gelling	Firm comparable with 0.5% agar gel
Color and clarity of ready medium	Light amber colored opalescent medium
Growth Promotion properties	Best at ≤ 100 CFU at 30-37 °C for 18-72 h
Indicative properties	Optimum at ≤ 100 CFU at 30-37 °C for 18-48 h
Negative control	Performed using sterile distilled water

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**Different Microbial Response: Cultural characteristics observed after incubation at 30-35°C for 18-48 hours in an anaerobic condition.**

Organism	ATCC	Inoculum	Growth
<i>Clostridium sporogenes</i>	11437	50-100	Luxuriant
<i>Clostridium perfringens</i>	3624	50-100	Luxuriant
<i>Clostridium novyi</i>	7659	50-100	Luxuriant
<i>Bacteroides vulgatus</i>	8482	50-100	Luxuriant
<i>Bacteroides fragilis</i>	23745	50-100	Luxuriant
<i>Lactobacillus fermentum</i>	9338	50-100	Luxuriant

**Storage and Shelf Life:** The product is highly hygroscopic; keep the container tightly closed at all times and store it properly as per the conditions mentioned on the label. The declared expiry is valid only when stored as per the conditions mentioned on the label. Note: Sterilize media immediately after reconstitution.

**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). 11<sup>th</sup> Edition. Difco Laboratories., Division of Becton Dickinson and Company, Sparks, Maryland, USA.
3. Indian Pharmacopoeia, (2018), Govt. of India, the Controller of Publication, New Delh
4. Rand, M. C., Arnold E. Greenberg, and Michael J. Taras, (1976), Standard methods for the examination of water and wastewater. Prepared and published jointly by American Public Health Association, American Water Works Association, and Water Pollution Control Federation.
5. The Japanese Pharmacopoeia, 17<sup>th</sup> Ed. (2016), The Ministry of Health, Labour And Welfare
6. The United States Pharmacopoeia, (2014), The United States Pharmacopoeial Convention. 12601 Twinbrook Parkway, Rockvukke, MD 20852.

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